UNIVERSITY of TASMANIA

Behaviour, Ecology and Social Organisation in *Liopholis whitii*: Insights into the Evolutionary Origins of Sociality

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Declarations

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Statement of Co-Authorship

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Abstract

Sociality is one of the most captivating properties of animal life, encompassing a remarkable diversity of behaviour. As well as varying greatly in form and function, social organisation is taxonomically widespread, indicating convergent benefits of group living. The centrality of social interactions in many ecological processes also makes social organization crucial to our understanding of a range of evolutionary principles. Despite this, both the mechanisms by which natural selection has led to diversity in social systems, and the conditions promoting the initial emergence of group living, remain unclear. If we are to understand how sociality emerges and diversifies over evolutionary time we need an integrated approach that identifies the causes and consequences of variation in social and mating behaviour in ecological settings, and relate this variation to the selective forces modulating social complexity.

Reptiles have recently been highlighted as a valuable system to address questions about the evolution of sociality. One lineage in particular, the *Egernia* skinks of Australia, display considerable variation in social organization, with species representing a continuum of social complexity from solitary living to large communal family groups. This variation in social complexity is matched by parallel variation in rates of genetic monogamy, implicating mating behaviour in the evolutionary forces driving diversity in social traits. Studies suggest that diversification in social and mating behaviour across the group are the result of variation in ecological conditions, specifically variation in the distribution and availability of suitable habitat. However experimental studies evaluating the ecological context dependence of social and mating behaviour are lacking. My PhD had two primary aims. First, to experimentally evaluate the influence of key habitat characteristics on the social and mating behaviour of a family living *Egernia* skink, *Liopholis whitii.* Second, to translate this mechanistic understanding of behavioural variation to the processes underlying diversification of social organization more generally. This allowed me to connect processes occurring across levels of biological organization, contributing to a more holistic understanding of the emergence, maintenance and diversification of social living.

Section I

The first section of my PhD experimentally evaluated the ecological context dependence of social and mating behaviour in L. whitii. Previous research has shown considerable variation in the social and reproductive strategies adopted by different adults, with ecological conditions thought to be an important driver of variation. However, to what extent specific habitat characteristics affect the expression of these strategies is unknown. I used two large-scale captive population experiments to examine i) how the distribution of a key resource, crevice sites used as nesting habitat, shapes social and mating behaviour and ii) how resource availability influences offspring dispersal and opportunities for parent-offspring interaction. I achieved these aims through extensive observations of individual space use and behavioural interactions in a replicated experimental design, utilizing molecular methods for paternity assignment of offspring. Results from the first experiment show that when crevice sites were aggregated, adults maintained larger home ranges and overlapped more individuals of the opposite sex, resulting in an increased occurrence of polygynous social groups. Despite this, aggressive female territoriality imposed upper limits on polygyny by inhibiting female-female home range overlap and restricting male opportunities for monopolization of multiple females. These results help explain both the presence of variation in social and mating behaviour in natural populations of L. whitii, but also the relative rarity of polygyny as a male mating strategy under natural conditions. This experiment also revealed that the probability of a female engaging in extra-pair mating increased with the number of males overlapping her core home range area, indicating considerable flexibility in female mating behaviour contingent on local social density. This result emphasizes the often-overlooked importance of ecological conditions on female promiscuity, a key driver of evolutionary transitions in social complexity.

The second phase of this experiment examined the effect of habitat distribution on parent-offspring associations, revealing greater levels of parent-offspring overlap when habitat was aggregated. This pattern appeared to result from greater competitive exclusion among adults when crevice sites were centrally aggregated, forcing subordinates to occupy satellite crevices and ultimately reducing the number of vacant crevice sites available to offspring. Associating with parents had significant benefits for offspring in terms of enhanced growth and body condition, despite no discernable cost

to parents of tolerating offspring overlap. Such conditions are expected to stabilize the expression of parent-offspring interactions and may even facilitate the evolution of more complex forms of parental care. However, these results also suggest that family stability in L. whitii varies in response to habitat availability via the plastic expression of delayed offspring dispersal.

Therefore in the second experiment I manipulated the density of conspecifics in enclosures available for offspring dispersal, both to evaluate the influence of habitat saturation on dispersal costs and directly test the tendency for offspring to delay dispersal under ecological constraints. Offspring showed a willingness to disperse when conspecific density in receiving habitat was low and habitat vacancies were available, but delayed dispersal at high densities when unoccupied habitat was limited. Furthermore, offspring attempting to settle high-density habitat were less successful, experiencing reduced survival compared to settlers in low-density habitat. Dispersal movements were also more costly under these conditions suggesting that dispersal costs were mediated largely by conspecific aggression; offspring that explored, but did not settle, high-density enclosures had reduced body condition and a greater risk of mortality. Importantly, those offspring that delayed dispersal and formed positive social associations with their mother showed superior increases in body condition over the course of the experiment. Taken together these results establish an important link between ecological conditions, delayed dispersal and social organisation. Offspring of L. whitii behave flexibly in response to habitat constraints, maintaining facultative social associations with parents when suitable habitat is limiting. Thus, demographic and ecological factors directly affect both the costs of dispersal and the potential for beneficial interactions among kin, and have likely been central to the diversification of social organization across the Egernia lineage.

Section II

The second section of my PhD complements this experimental work, moving from an examination of the ecological mechanisms by which diversity in social and mating behaviour can arise, to a broader scale examination of the processes that influence the diversification of social traits across species. In this section I focused primarily on parental care. Complex forms of parental care have generally been assumed to be rare or even absent in lizards. Here I show that lizards (and squamates in general) exhibit all

but the most advanced forms of care found in other ectothermic taxa such as fish and amphibians, including nest construction, egg brooding and defence, neonatal attendance and prolonged post-partum parent-offspring associations. This functional and taxonomic diversity in care, combined with a well-resolved phylogeny of the squamates provides an excellent opportunity to address fundamental questions regarding the evolution of parental care. In particular, as social tolerance between overlapping generations is a defining feature of many social groups, the presence of facultative post-partum parent-offspring associations in a range of species allows an investigation of the factors that predispose certain lineages to evolve sociality. Prolonged parent-offspring interactions should be more likely to evolve when parents regularly encounter their offspring. Giving birth to live young or attending eggs should increase opportunities for such encounters, and may therefore promote a transition from solitary to group living. Squamates provide a unique opportunity to test this hypothesis since, unlike in mammals and birds, live bearing and egg attendance have evolved many times in different lineages. To address this hypothesis we used a series of phylogenetically controlled tests to examine the correlated evolution of viviparity, egg attendance and social grouping. We found evidence for intergenerational social groupings in 100 species across 36 families. Groupings always involved adult females and have evolved considerably more often in viviparous species than in oviparous species, including oviparous species that attend their eggs. This is consistent with the hypothesis that stable social groupings in squamates emerge from tolerance of offspring rather than from aggregation of unrelated individuals. Indeed, phylogenetic reconstructions indicate that all but 2 of the 16 independent origins of stable social grouping have occurred in viviparous lineages, highlighting live bearing as an important precursor in the evolutionary origins of social organization in the lineage.

Conclusions

This thesis provides the first robust tests of the ecological context dependence of several key aspects of social and mating behaviour in *L. whitii,* providing supporting evidence for the influence of environmental heterogeneity on the evolutionary diversification of social organisation across the *Egernia* lineage. From this mechanistic perspective, I demonstrate how an understanding of processes driving individual level variation in social behaviour may be scaled up to ask questions about the evolutionary

origins of sociality itself. This approach demonstrates the power of combining experimental and comparative methods in a hypothesis driven framework to connect species level biology with phylogenetic patterns in behavioural traits. The results generated from this study provide exciting new avenues for future research. In particular, the analytical framework established here invites more focused comparative studies across the *Egernia* group. While many species conform well to our predictions of ecological context dependence in the evolution of social organisation, some patterns remain unexplained. For example, the convergence of social systems among *Egernia* species that occupy distinct habitat types (e.g. *E. stokesii* and *E. frerei)* and, conversely, divergence in social organisation between those inhabiting seemingly similar habitats (e.g. *L. striata* and *L. kintorei*), demonstrates the limited generality of our predictions in explaining the diversification of social organisation across the group. Targeted comparative studies incorporating climatic, environmental and life-history data could shed light on these patterns and continue to refine our understanding of social evolution in this fascinating and charismatic group of Australian reptiles.

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Chapter One

General Introduction

"Some are gregarious, some are solitary, whether they be furnished with feet or wings or be fitted for a life in the water…. And of the gregarious, some are disposed to combine for social purposes, others to live each for its own self."

ᔓ Aristotle, The History of Animals. Fourth century BCE ᔐ

The occurrence of social aggregations in animals had earned the fascination and reverence of humans long before Aristotle wrote his seminal works. Cave paintings from France, Australia, Indonesia and South America, some dating back more than 40000 years, depict herds of animals, often in close association with humans (Aubert et al. 2014; David and Lefrère 2014). Some portray animals interacting with human figures, others show animals intermingled with stencils of human hands: a union of symbols that seem to both acknowledge and venerate our shared habit of sociality.

As well as encompassing some of the most majestic and spectacular forms of animal behaviour, the widespread occurrence of sociality across the animal kingdom raises the fundamental question of why animals live together. In contemporary scientific thought, this enquiry has generated considerable interest in the conditions that favour the evolutionary maintenance of sociality and led to great advances in our understanding of the benefits of social living. However while the benefits of sociality have become clearer, the mechanisms by which sociality initially emerged still presents one of the most complex and contentious questions in biology.

Sociality: Causes and Consequences

Sociality is remarkably diverse, ranging from ephemeral associations between breeding adults, to mating partnerships maintained across years, stable family groups with overlapping generations, and complex eusocial colonies featuring morphologically distinct castes, sterile workers and intricately specialized divisions of labour (Krause and Ruxton 2002). Complex social systems also occur in a wide variety of taxa. For example, cooperative-breeding systems, in which individuals forgo reproduction to help raise siblings at the nest are common among birds (Koenig and Dickinson), but also occur in mammals (Clutton-Brock 2006) and crustaceans (Duffy and Macdonald 2010). The presence of similar social systems in such disparate groups indicates convergent benefits of sociality. Indeed, comparative phylogenetic analyses show that social living has arisen independently many times and within many different taxonomic groups (Lubin and Bilde 2007; Duffy and Macdonald 2010; Lukas and Clutton-Brock 2013), suggesting that factors which drive selection for particular modes of living may be shared across taxa. As a result, there has been a wealth of studies attempting to identify common causal factors responsible for the diversification of social systems (Hughes et al. 2008; Boomsma 2009; Hatchwell 2009; Cornwallis et al. 2010; Gardner et al. 2015). While these studies emphasize the importance of relatedness among group members, both the mechanisms by which natural selection has led to this diversity and the specific ecological conditions promoting the initial emergence of group living remain unclear.

A defining characteristic of sociality is the occurrence of cooperative behaviour among conspecifics. These behaviours range in complexity from simple tolerance of individuals accessing a shared resource (Chapple 2003; Dickinson and McGowan 2005) to vigilance or defensive behaviour that poses considerable risk to the actors involved (e.g. Griesser and Ekman 2005). Cooperative behaviours can be maintained in a population by mutualistic benefits if each member of the group stands to benefit from the interaction, or by direct reciprocity if conditions prevent the invasion of cheaters (Nowak 2006). However cases in which animals appear to act altruistically toward other group members, displaying behaviours that provide no apparent fitness benefit to the actor and in many cases involve obvious fitness costs, require a different explanation; namely, that individuals may gain fitness indirectly by cooperating preferentially with relatives (Hamilton 1964a; Hamilton 1964b).

The theory of inclusive fitness reveals that the strength of selection on a given gene must be thought of in terms of that gene's effect on the total number of gene copies passed onto the next generation. When framed in terms of a gene coding for a cooperative behaviour, this is the sum of gene copies that are transferred directly through the production of offspring and indirectly via the effect of that behaviour on the fitness of other individuals in the population that also share the gene (West et al. 2002). Because closely related individuals share genes that are identical by descent, altruistic behaviours can evolve when the benefits to reproduction or survival conferred to close relatives by acting 'altruistically' results in more copies of those genes being passed onto subsequent generations than if individuals had acted selfishly. This elegant principle is captured by the simple equation in Hamilton's Rule:

$$rB > C \qquad (1)$$

Which holds that cooperative behaviours will be selected for when the level of relatedness between the actor and recipient of a behaviour *r*, multiplied by the benefit conferred to the recipient *B*, is greater than the cost to the actor *C*, in terms of reproductive success.

Hamilton's Rule emphasizes the importance of feedbacks between behaviour and ecology, because the ecological context of an organism will dictate the genetic structure of a population (r), as well as how beneficial (B) or costly (C) behaviours will be to the individual. In reality these feedbacks can be incredibly complex, depending also upon developmental, genetic and behavioural constraints imposed by phylogenetic affinities, life history characteristics, fitness landscapes, sexual conflict and modes of information transfer between generations (Alonzo 2010; Shea 2013; Rehan and Toth 2015). Thus, ecology acts to contextualize the terms in Hamilton's rule and must be considered to integrate the ecologically responsive organism into genetic theories of social evolution. Despite this, there have been surprisingly few studies that attempt to clarify the proximate causes of variation in relatedness among group members, or the costs and benefits of cooperation in ecological settings. As a result we still have a poor understanding of the underlying ecological mechanisms that promote variation in behaviours relevant to inclusive fitness.

To close this gap in current knowledge and refine our understanding of the evolutionary maintenance of sociality targeted empirical studies are necessary. Research investigating the general ecological conditions and specific environmental factors that promote the initial emergence of social behaviour is particularly scarce. Indeed, focusing on the power of inclusive fitness to describe the maintenance of sociality appears to have come at a cost to our understanding of its origins (Linksvayer and Wade 2005; Gordon 2013). After all, social behaviour relies first and foremost on regular encounters between conspecifics; only when ecological conditions promote stable social associations between conspecifics will the stage be set for the development of more complex social interactions. The evolutionary elaboration of social behaviour may then precede either through mutualistic (egalitarian) or inclusive (fraternal) benefits, providing sociality increases the net fitness of interacting individuals (Kokko et al. 2001; West et al. 2002; Nowak 2006). Therefore a theory of social evolution requires an understanding of both the specific ecological conditions that promote stable social interactions, and the mechanisms by which selection drives divergence in social traits once they arise.

For most animals, social organization is intimately linked with mating behaviour. Indeed, reproduction necessitates social interaction between individuals, and patterns of mating behaviour typically reflect the structure of animal societies (Andersson 1994; Arnold and Duvall 1994). This means that by understanding factors that influence mating behaviour at the individual level we gain insights into the mechanistic basis for evolutionary divergence in social traits. Emlen and Oring (1977) were among the first to recognize this, proposing that habitat characteristics will be instrumental in this process. Specifically, the distribution of resources necessary for reproduction will directly influence the dispersion of potential mates, and therefore the nature of social interaction among conspecifics and the cost-benefit trade off of different mating strategies at the population level. Thus, variation in resource distribution provides a mechanistic explanation for variation in social and mating behaviour, but also helps explain the reoccurrence of similar mating systems across taxa.

This link is encouraging, as it develops a framework within which to connect the processes that produce variation with patterns of variation we see across species (*sensu* Weber and Agrawal 2012), and has received strong support from natural population studies on a range of taxa (Davies and Lundberg 1984; Carrete et al. 2006; Stradiotto et

al. 2009). However most studies are correlative, with surprisingly little research using experimental manipulations of resource distribution to actually test the ecological context dependence of social and mating behaviour (although see Lucia et al. 2008). Conversely, the few comparative studies that have investigated broad scale environmental correlates of social behaviour stop short of evaluating causality in the ecological mechanisms suggested by their results (Arnold and Owens 1999; Jetz and Rubenstein 2011; Majer et al. 2013). Thus, the integration of experimental and comparative studies is a crucial step toward advancing our understanding of social evolution.

To achieve this aim we need to identify the ecological conditions under which social behaviour emerges, how variation in these conditions shape the strength and nature of social interactions, and then go on to test how this reflects the diversification of social behaviour across species. This requires an experimental approach and, therefore, study systems that are amenable to ecological manipulations. Furthermore, in order to conduct a meaningful investigation of the evolutionary origins of sociality, we need to focus on a group of organisms in which social behaviour is a derived and facultative state. Identifying ecological conditions that promote or suppress social behaviour in such a group would provide direct evidence for the role of ecology in the initial emergence of sociality as well as valuable insights into the specific mechanisms mediating this variation.

Sociality in the Egerniinae

Reptiles are increasingly being highlighted as an alternative study system to address questions about the evolution of sociality (Davis et al. 2011; Doody et al. 2012; Gardner et al. 2015). Indeed, many characteristics of reptiles make them ideally suited for evolutionary studies: 1) Sociality is a derived state; 2) social groupings are typically facultative, which means they provide the opportunity to investigate conditions that cause a switch in the expression of social behaviour; 3) sociality shows signs of phylogenetic structure, with social organisation particularly common in a few select lineages; however, 4) these lineages are taxonomically diverse, suggesting multiple independent origins of social living. Finally, 5) reptiles are highly amenable to experimental studies; they take well to captivity and behave naturally under

experimental conditions. These traits make reptiles an excellent study system to investigate the evolutionary origins of social behaviour. One group that shows particular promise in this regard is the Egerniinae, or Egernia skinks, of Australasia.

The Egerniinae (hereafter *Egernia*) are a group of skinks containing 58 species distributed among 8 genera (*Bellatorias, Corucia, Cyclodomorphus, Egernia, Liopholis, Lissolepis, Tiliqua, Tribolonotus*) that occupy a diverse range of ecological niches across Australia and Papua New Guinea. Within Australia, species inhabit a variety of environments, encompassing all major climatic and floristic zones, and show considerable variation in life history traits (Chapple 2003). Since the discovery of long-term socially monogamous pair bonds in the sleepy lizard, *Tiliqua rugosa* (Bull 1988), research focused on the group has revealed the presence of complex sociality in over 20 species, typically characterized by male-female pair bonds, delayed juvenile dispersal, and prolonged parental association with offspring (Chapple 2003; Gardner et al. 2015). However the stability and complexity of social groups varies markedly across the lineage, with species representing a continuum of social complexity from solitary living to nuclear families to large communal family groups (Chapple 2003). Parental care behaviour varies accordingly, from being absent (e.g., *Tiliqua rugosa*: Bull and Baghurst 1998), to tolerance of a single offspring or cohort of offspring (nuclear families, e.g., *Liopholis whitii*, *Egernia frerei*: Fuller et al. 2005; While, Uller, and Wapstra 2009a), to social groups of multiple adults tolerating multiple cohorts of offspring (communal groups, e.g., *Egernia cunninghamii*, *Egernia saxatilis*, *Egernia stokesii*: Gardner et al. 2001; O'Connor and Shine 2003; Stow and Sunnucks 2004).

Both proximate and ultimate hypotheses for the formation of group living in Egernia have been developed, focusing on the mechanisms driving the formation of social groups and the benefits of group living, respectively. For the latter, group living is suspected to confer benefits via enhanced vigilance (Lanham and Bull 2004), or via offspring protection from conspecific aggression (O'Connor and Shine 2004; Langkilde et al. 2007) or predation (Masters and Shine 2003). From a proximate perspective, convergence of social complexity among species that occupy certain habitat types has led to the suggestion that environmental variation has been a major contributor to the evolutionary diversification of social organization across the group. Specifically, that the structure and distribution of habitat has important implications for space use among individuals, including opportunities for aggregation and the optimal group size

supported by retreat sites (Duffield and M. Bull 2002; O'Connor and Shine 2003; Michael and Cunningham 2010). Indeed, those species showing the largest and most complex social groups (i.e. *E. cunninghamii, E. stokesii and E. saxatilis*) all occupy isolated rocky outcrops providing large communal crevice sites and limited opportunities for dispersal between outcrops. However, natural population studies attempting to evaluate these hypotheses have yielded equivocal results (Osterwalder et al. 2004; Fuller et al. 2005; Gardner et al. 2007; Michael and Cunningham 2010).

One of the challenges of understanding the evolution of mating and social systems is that that the many traits that compose these systems are inherently interconnected. In order to understand and predict the evolution of any given trait we must therefore consider relevant co-evolutionary feedbacks between traits that compose these systems (While, Uller, and Wapstra 2009b; Alonzo 2010). For example, recent comparative analyses indicate that the evolution of social monogamy preceded and indeed facilitated the evolution of male care in mammals (Lukas and Clutton-Brock 2013; Opie et al. 2013), ostensibly by allowing greater male investment through enhanced paternity assurance (Woodroffe and Vincent 1994; Alonzo 2010). Once male care emerges, the benefits conferred by this care may even feedback to constrain variation in mating behaviour for both sexes, contributing to the evolutionary maintenance of monogamy (While, Uller, and Wapstra 2009b).

Unlike other systems, reptiles are well suited to studies attempting to disentangle co-variation between social and mating traits, both because social traits are relatively simple and because reptiles are highly amenable to the experimental manipulations necessary to isolate these effects. Recent research has even begun to characterize these feedbacks in one particular Egernia skink, *Liopholis whitii*. Ongoing natural population studies of *L. whitii* suggest that a behavioural feedback between the sexes maintains selection on social monogamy (While, Uller, and Wapstra 2009b). However experimental studies are required both to demonstrate causality in the feedback between social and mating traits and determine how local ecological conditions shape the nature of social interactions.

Study Organism

Liopholis whitii is a medium sized (<100mm snout-to-vent length) viviparous skink found in dry habitats throughout south-eastern Australia. This species lives in nuclear family groups based around a stable socially monogamous adult pair and offspring that delay dispersal for up to a year to reside within the parental territory. Specifically, in each monogamous pair both male and female *L. whitii* tolerate offspring within their territory, providing offspring that delay dispersal access to limiting resources and protection from conspecific aggression (Sinn et al. 2008; While, Uller, and Wapstra 2009b). Females also engage in extra-pair copulations, with approximately 30% of offspring sired by males outside the pair bond. Promiscuity is selected for via the positive effects it has for reproductive success in males and, perhaps, inbreeding avoidance in females (While et al. 2011; While et al. 2014; Bordogna et al. 2016). Crucially, studies suggest that males are able to assess whether or not they are the father of offspring (via olfactory kin recognition) and alter their investment in care accordingly (While, Uller, and Wapstra 2009b). Long-term natural population data show that, within each family unit, extra-pair offspring are found outside the home range of the resident male (i.e. cuckolded male) significantly more often than within-pair offspring (While, Uller, and Wapstra 2009b), suggesting that males punish female promiscuity by actively excluding extra-pair offspring from their home range. This results in a behavioural feedback in which rates of extra-pair paternity negatively co-vary with male tolerance of offspring at the population level, influencing variation in the composition of social groups (While, Uller, and Wapstra 2009b; While et al. 2011).

Importantly, While et al. (2009) point out that these traits will be heavily influenced by local ecological conditions. Indeed, although basic features of the social and mating system of *L. whitii* appear relatively consistent between populations (Chapple 2005; Chapple and Keogh 2005; Chapple and Keogh 2006; While, Uller, and Wapstra 2009a), there is still considerable temporal and spatial variability in behaviour (While, Uller, and Wapstra 2009a; While et al. 2011). For example, rates of polygyny, extra-pair paternity and offspring tolerance vary within and between populations and across years (Chapple and Keogh 2005; While, Uller, and Wapstra 2009b), implicating local environmental conditions as a mediator of individual behaviour. From a mechanistic perspective, variation in resource availability or conspecific density will

dictate mating opportunities by influencing encounter rates (Uller and Olsson 2008), which in turn will define the costs and benefits of different mating strategies (While, Uller, and Wapstra 2009b). Resource availability will also influence the costs and benefits of delayed dispersal for offspring and prolonged care for both males and females (Komdeur 1992; Kokko and P. Lundberg 2001; Alonzo and Klug 2012). Over evolutionary time scales consistent differences in ecological conditions may therefore contribute to the maintenance of social complexity and fixation or further elaboration of social traits, as both the opportunities for social interaction and the fitness consequences of different social behaviours will be determined by ecological context. However the effects of specific habitat variables on behaviours with known evolutionary implications, such as male and female mating behavior and delayed offspring dispersal, are yet to be investigated.

Research Objectives

The overarching aims of this thesis are to 1) empirically evaluate key feedbacks between behaviour and ecology that contribute to the evolutionary maintenance of social organisation in *L. whitii* and 2) use this mechanistic understanding of behavioural variation to inform comparative analyses aimed at identifying the ecological and life-history factors responsible for the emergence of complex social organisation across the squamate reptiles. An emergent aim of the thesis was to show how an understanding of these links are valuable to more general insights into the mechanistic processes that drive not only the maintenance and diversification of complex social behaviour across taxa, but also its initial emergence from more primitive social systems. To this end I chose an approach that combined large-scale captive population and laboratory experiments with in-depth literature reviews and phylogenetic comparative analyses to establish causal relationships between ecological conditions, behavioural responses and opportunities for selection on social traits.

By combining these approaches my thesis answers a growing recognition of the value of reptiles as alternative model systems in evolutionary ecology (Doody et al. 2012; Gardner et al. 2015). In particular, I turn to reptiles to address evolutionary questions that cannot easily be answered by studies on birds and mammals. Focusing my experimental work on *L. whitii*, a reptile with simple and facultative sociality,

provides an analogue for the socio-ecological feedbacks operating early in the evolution of family living. This allows me to address questions, not only about the diversification of social organisation across one of Australia's most distinctive faunal groups, but also about the very origins of sociality itself.

Thesis Presentation

This thesis comprises five data chapters divided into two sections associated with the key research objectives outlined above. All chapters have either been accepted for publication in relevant scientific journals or are currently in review for publication. As such each chapter is written as a stand-alone piece of work and may incur some repetition, particularly in terms of methods describing animal collection, husbandry, species descriptions and experimental protocols. As the chapters presented have been modified from manuscripts prepared for submission, each chapter may also vary slightly in formatting due to the specific requirements of each journal. Two chapters (chapters 4 and 5) include papers for which I am second author; however as the study described in chapter 4 was conducted under my supervision, using data collected from the second phase of the experiment presented in chapter 3 and as I contributed directly (and in the case of chapter 5, equally) to data collection, statistical analyses and manuscript preparation, they have been included. Finally, opportunities afforded to me during my PhD have resulted in collaboration on publications that do not relate directly to the content of this thesis, but were nonetheless published during my candidature at the University of Tasmania. These works are included as appendices.

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Section I

Chapter Two

Resource Distribution Mediates Social and Mating Behavior in a Family Living Lizard

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Abstract

The distribution of resources should influence mate availability and the costs and benefits of pursuing different mating strategies. Where resources are dispersed, males may be constrained in the extent to which they can monopolize more than one partner, resulting in social and genetic monogamy. There is abundant correlational evidence that resource distribution influences social and mating systems, but experiments that demonstrate a causal link between these variables are relatively rare. Here we used a replicated experiment involving 160 animals to examine how the distribution of a key resource, crevice sites used as nesting habitat, shapes social and mating behavior of a family living lizard, *Liopholis whitii*. The distribution of crevice sites had significant effects on several important aspects of the social and mating system. When habitat was aggregated, adults had larger home ranges and overlapped with more individuals of the opposite sex, resulting in increased opportunity for social polygyny. Aggressive female territoriality appears to impose upper limits on opportunities for polygyny by restricting female-female home range overlap. Despite this, males in aggregated habitats still formed polygynous social groups more often than males in dispersed habitat. Aggregated habitat also increased the opportunity for sexual selection, resulting in greater variance in male reproductive success and a steeper Bateman gradient compared to males occupying dispersed habitat. These effects were independent of the increase in social polygyny. Overall, our study is consistent with the hypothesis that habitat structure is fundamentally important to the evolution of social and mating systems.

Introduction

Understanding processes that mediate the evolution of social living is a key objective in evolutionary ecology. In many systems, variation in social organisation is closely associated with spatial and temporal heterogeneity in resources, such as food and shelter. Resource distribution and availability were originally suggested to explain the evolution of spacing patterns and territoriality in birds (Brown 1964), but now underlie theories for the evolution of cooperative breeding, year-round residency, delayed dispersal, reproductive skew and sexual traits in a wide variety of taxa (e.g. Koenig et al. 1992; Komdeur 1992; Emlen 1995; Hatchwell and Komdeur 2000; Kokko and Ekman 2002; Clobert et al. 2009; Cornwallis and Uller 2010). Resource distribution is also important in dictating which mating strategies are most successful by affecting the reproductive opportunity for males and females and the costs and benefits of acquiring partners (Shuster and Wade 2003; Oh and Badyaev 2006). For example, when females seek out particular resources, such as food or nest sites, the distribution of these resources should determine the potential for males to economically monopolize single versus multiple mates (Emlen and Oring 1977).

Empirical studies have demonstrated that resource distribution does indeed co-vary with social organization across a broad range of taxa (mammals: Campbell et al. 2006, Lukas and Clutton-Brock 2013; Rémy et al. 2013; fishes: Thompson et al. 2006; Przybylski et al. 2007; Wong et al. 2012; birds: Leisler et al. 2002; Covas et al. 2004; Halupka et al. 2014). For example, home range overlap of male and female yellow-necked mice, *Apodemus flavicollis,* co-varies with food availability (Stradiotto et al. 2009) and the formation of polygynous mating groups in the dunnock, *Prunella Modularis,* is associated with the density and proximity of suitable foraging patches (Davies and Lundberg 1984). The majority of studies to date have focused on birds and mammals. While these systems provide valuable insights into how resource distribution influences the maintenance and diversification of social organization, the near ubiquity of sociality in these taxa means that they tell us little about the role these factors may play in the initial origins of social organization. To address this shortcoming we need to identify systems in which sociality is simple and facultative as they will provide insights into the role resource distribution may have played in the initial emergence of group living.

Lizards are increasingly being recognized as valuable alternative systems in socio-ecological research (Doody et al. 2009; Gardner et al. 2015; Leu et al. 2016). Unlike birds and mammals, most lizards do not provision their young postpartum, alleviating sexual conflict that selects for male adherence to a monogamous mating strategy under the constraints of obligatory paternal care (discussed in Emlen and Oring 1977). This feature allows for a more direct interpretation of the influence of ecological variation on mating strategies and the consequences of this for evolutionary transitions to more complex forms of sociality (While et al. 2014). Lizards are also highly amenable to experimental manipulation (Le Galliard et al. 2005; Warner and Shine 2007; Keogh et al. 2013; While et al. 2015) and, combined with genetic methods of paternity assignment, make it possible to identify the causal relationships between resource availability and the social and mating system.

Among the lizards, skinks of the *Egernia* group (Egerniinae) display considerable variation in social and mating organization. Species range from being solitary, to forming long-term pair bonds and family groups, to complex multi-generational social groups that persist between years {Chapple:2003vb, Gardner:2015es}. Some studies suggest that variation in social and mating organization is a direct result of variation in the availability and structure of crevice sites, the refugia around which the majority of an individual's daily activities take place (Duffield and Bull, 2002; Chapple, 2003; O'Connor and Shine, 2003; While et al., 2009a). However, current empirical evidence for a link between crevice site distribution and social systems in *Egernia* is equivocal. For example, manipulation of crevice site availability in *E. striolata* altered adult pair bonding, with more pairs forming when crevice availability was low (Lancaster et al., 2010), whereas in *E. stokesii* crevice site abundance had no effect on the social group structure (Gardner et al. 2007). To address the role that crevice site availability may have played in the emergence and diversification of social organization in *Egernia*, we therefore need to isolate alternative causative factors in well-replicated, manipulative experiments.

Here we experimentally tested how the structure and availability of habitat (crevice sites) influences the mating and social behavior of *Liopholis whitii* (formerly *Egernia whitii*). *Liopholis whitii* live in nuclear family groups comprising of a long-term adult pair and a single cohort of offspring (While et al. 2009). However, this species also displays considerable within population variation in social and mating strategies, with

both monogamy and polygyny occurring at the social and genetic level (While et al. 2009). Approximately 70% of adults exhibit social monogamy characterized by stable long-term pair bonds with the remaining ~30% forming polygynous social groups whose composition may vary across years (While et al. 2009). Similarly, while genetic monogamy is the norm, approximately 30% of offspring are the result of genetic polygyny (e.g., extra-pair paternity). The ability of a male to acquire both social and genetic polygyny has significant implications for his reproductive success (While et al. 2011). Therefore, this species offers an excellent opportunity to explore the extent to which variation in individual social and mating strategies depend on the structure and availability of habitat resources (e.g., crevice sites).

We housed captive populations of *L. whitii* in large semi-natural enclosures with either an aggregated or dispersed coordination of available crevice sites. We predicted that aggregating resources would increase the extent of inter-sex overlap, increasing the frequency of both social and genetic polygyny, and the potential for sexual selection on male phenotypic traits. If multiple paternity in *L. whitii* is controlled largely by male-female encounter rates (sensu Uller and Olsson 2008), we also predicted that aggregating resource patches would increase rates of multiple and extra-pair paternity among females.

Materials and Methods

Study species

Liopholis whitii is a medium sized (<100mm snout-to-vent length (SVL)) viviparous skink found in dry habitats throughout southeastern Australia. Morphology and life history traits vary geographically (see Chapple and Keogh 2005). In Tasmania, males and females are sexually monomorphic, become reproductively mature at approximately 3 years, and have an overall lifespan of 9–10 years (G. While, unpublished data). Reproduction occurs annually, with mating occurring during the austral spring (September–October). Gestation spans 3–4 months with births occurring in the Austral summer (January-February) (While et al. 2007).

Animal capture and husbandry

During August 2013 we captured 80 males and 80 females from a population at Orford on the east coast of Tasmania, Australia (42°57'S, 147°88'E) using fishing and noosing techniques (Dodd 2016). Upon capture animals were secured in canvas bags and placed in a cooler during transport back to University facilities. In the laboratory we measured individuals for SVL, total length, head length and head width (±1 mm), mass (±1 mg) and determined sex by hemipenis eversion. We took a tail tip sample from every individual for genetic analysis (see below). Animals were toe-clipped for unique identification.

Animals were housed individually in temperature- and light-controlled rooms, with room lights set to a natural (12:12) day-night cycle. Terraria (30x60x40) were made from opaque plastic and contained ~5cm of paper-based cat litter, food and water dishes, a shelter at one end and a basking rock at the other. Basking lamps were set to come on 1 hour after ambient lights came on (simulating sunrise) and turn off 1 hour before they went out (sunset). This provided a thermal gradient of 17-40°C in the terraria, allowing animals to bask to their preferred body temperature of 34°C (Bennett and John-Alder 1986). Water was provided *ad libitum* and animals were fed every two days on mealworms dusted with mineral supplement and periodically with pureed fruit. Animals were held under these conditions for ~3 weeks, before being randomly allocated to treatment groups and released into the experimental enclosures. After random allocation we confirmed that both sexes were matched for SVL and body mass across treatment groups using a Gaussian GLM with treatment, sex and the treatment by sex interaction term as fixed effects (Table A1.1).

Experimental Protocol

Enclosure set up

We conducted the experiment from September 2013 to February 2014 at the University of Tasmania's Cambridge farm facility. We used 16 large (8x8m) outdoor enclosures constructed from 40cm high tin walls and the entire compound (30x70m) was enclosed with bird exclusion netting (Figure A1.1). Each enclosure contained five 1x1m wooden pallets representing high quality habitat patches and 4 small satellite patches consisting of two cement blocks representing low quality habitat. Each pallet had ~100L of

sand/gravel mix placed in the center to provide burrowing substrate and 6 cement blocks arranged on top to provide a basking site with each major aspect. Low quality patches were not provided with any burrowing substrate and were distributed at equal distances from high quality habitat patches in both treatments (see below). We installed 4 drink stations in each enclosure and periodically supplemented available forage by scattering an equal amount of live food (mealworms and crickets) throughout each enclosure.

We manipulated the distribution of crevice sites within our enclosures in a two-treatment design, providing 8 replicates of both an aggregated and dispersed resource distribution. In the aggregated treatment, habitat patches containing high quality crevice sites were clumped in the center of the enclosure, while in the dispersed treatment high quality sites were distributed uniformly throughout the enclosure (Figure A1.2). Thus, all enclosures had an equal number of crevice sites but differed in the physical distribution of those sites.

Behavioural observations

In mid-September 2013 we introduced 5 males and 5 females into each enclosure. This represented slightly higher densities than occur in wild populations because we wanted to create competition for high quality habitat patches. We attached colored cloth stickers (Tesa, Hamburg, Germany) to the back of each individual indicating their sex and enclosure ID for rapid identification during observations (Figure A1.3). We collected data on adult locations and behavioural interactions twice a day, once in the morning (8am-12am) and once in the afternoon (3pm-6pm) on days suitable for lizard activity for three months. The location of each individual was recorded only once per session and observations of the same enclosure were at least three hours apart to avoid autocorrelation. In each session, an observer (BH) spent 10 minutes walking slowly around the perimeter of each enclosure, recording the physical position of each individual on enclosure maps and detailing any interactions (e.g. fights, chases, copulations). Once each enclosure had been observed, a further 20 minutes was spent walking throughout the entire enclosure complex, opportunistically collecting observations of any individuals not previously observed. The order in which enclosures were observed was randomized between sessions to avoid temporal effects. The behavioral interactions recorded ('chase', 'fight', 'basking close', 'contact' and

'copulation') were observed too infrequently to include as response variables in formal analyses. However, we were able to use 'contact' (male-female) and 'basking close' (male-female) interaction data to inform social pairings (see below). We defined 'basking close' as when a male and a female were observed basking within a body length of each other but were not observed to interact further. We defined 'contact' as any event where a male and female came into physical contact but did not engage in agonistic behavior or copulation (see also While et al. 2015; Heathcote et al. 2016).

Towards the end of gestation (mid-January), all animals were recaptured, brought back into the laboratory and housed as described above. The terraria of gravid females were checked daily for offspring. Upon discovering offspring, date of birth was recorded and the individual was temporarily removed from its mother to obtain weight (± 10 mg), SVL (± 0.5 mm) and total length (± 0.5 mm). Each offspring was then toe-clipped for unique identification and had a tissue sample taken (~5mm of tail tip, subsequently stored in 90% ethanol) for paternity analysis.

Estimating home ranges and overlap

We used least-squares cross validation (LSCV) in ArcView 9 (Esri 2004) to calculate 50% kernel home ranges areas (henceforth, HR size) for each individual using the positional point-data collected during observations. The median number of observations of an individual during our experiment was 16. Although 30 points is considered the minimum number of observations to reduce bias when generating home range kernels via LSCV (Harris et al. 1990), *L. whitii* are highly territorial and spend the vast majority of their time in or around a single burrow site. As a result, observations were generally tightly clustered around a specific location, making core home range areas easy to define and less prone to bias from random sampling (see Tinkle et al. 1962; Hews 1993). Therefore we decided to exclude from further analyses only individuals that had fewer than 8 observations (n=17). Because the number of observations recorded for the remaining individuals varied considerably (females: 8-29, males: 8-59), we also set a constant LSCV smoothing factor of 0.75 when generating kernels rather than allow ArcView to automatically assign a smoothing factor based on the data cloud of each individual. A smoothing factor of 0.75 was chosen because it was most effective at buffering against over-smoothing the kernels of individuals at the high end of the range of sample size as well as under-smoothing those at the low end (see Kie

2013). Each kernel was then individually checked against the data cloud to verify that it represented a realistic estimate of the core home range of individuals based on observations. In *L. whitii*, 50% kernels represent an individual's permanent shelter site around which it basks, forages and experiences the majority of its social interactions (Chapple 2003; While et al. 2009). Therefore, we estimated home range overlap as the number of conspecifics and percentage of the home range overlapped at the 50% kernel area.

Identifying Pair bonds

We defined pair bonds based on percentage of kernel overlap as in previous studies of *Egernia* group lizards (e.g. While et al. 2009). The male that overlapped the greatest percentage of a particular female's kernel HR was considered to be her social partner (see While et al. 2009). Where a male was identified as the social partner of multiple females using this criterion he was considered socially polygynous (n = 16). Where a male was not identified as the social partner of any females based on this criterion he was considered unpaired (n = 26). Males and females identified as pairs overlapped a considerably larger proportion of their home range compared to the average male-female overlap (see Table A1.2). We defined a female as not having a social partner if she did not overlap with any males (n = 11) or if percentage overlap with the most overlapping male was <10% and we had not observed any positive interactions or 'proximity' observations (defined as observations where both individuals were observed occupying the same habitat patch within an observation period) between the focal female and the most overlapping male (n = 1). In cases (n = 13) where two males overlapped the focal female to a similar extent (e.g., the two most overlapping males were separated by <10%), we assigned the male with the greatest number of positive interactions and proximity observations with the focal female as the social partner. When there were either none or an equal amount of interactions or proximity observations recorded between the female and both the conflicting males we chose to accept the male with the greatest overlap as the social partner. To confirm this assignment of pair bonds we also conducted a separate set of analyses in which we generated 10 dummy data sets by randomizing the assignment of conflicting males to each female and checked for consistency of results between models run on the randomized data sets.

Parentage Assignment

All individuals included in the study were genotyped for six microsatellite loci (EST1, EST2, EST4, EST12: Gardner et al. 1999; TruL12, TruL28: Gardner et al. 2008) using standard molecular techniques with DNA extracted from tail tip samples (see While et al. 2009; While et al. 2011 for further details). Paternity was assigned using the computer program CERVUS 3.0 (Marshall et al. 1998) using the following simulation parameters: 10,000 cycles, 95% of candidate parents sampled, 95% loci typed and a genotyping error rate of 1% (calculated in CERVUS from our data). The 'one known parent' option was used, with all adult males released into the same enclosure as the mother included as possible fathers. Paternity was assigned to the male with the highest male-female-offspring trio LOD score and the lowest number of mismatches (0 or 1) (e.g., Chapple and Keogh 2005; While et al. 2009; While et al. 2014). Because there were only five possible fathers for any individual offspring within an enclosure, paternity could be assigned with high confidence in the majority of cases and 79 out of 82 offspring were included in analyses. By comparing the genetic father of offspring with the social father (assigned by greatest kernel overlap with the mother - see above), we could then determine whether offspring were the result of within- or extra-pair copulations. Using this data we quantified male reproductive success (RS) as the total number of offspring sired based on paternity analyses. We also quantified male mating success (MS) as the number of genetic partners a male sired offspring with. Female reproductive success was quantified based on her litter size.

Statistical analyses

We conducted all statistical analyses in R (Version 3.1.2: Core Development Team 2014), using linear mixed models (LMMs) and generalized linear mixed models (GLMMs) fit by the 'lmer' (for Gaussian distributed response variables) and 'glmer' (for binomial and Poisson distributed response variables) functions in the lme4 package (Bates et al. 2014). Parameter estimates were derived using Laplace approximation. For LMMs, the significance of fixed effects are reported based on *F*-tests with degrees of freedom approximated by the Kenward-Rogers method in the package lmerTest. For GLMMs, the significance of fixed effects are reported from likelihood-ratio tests obtained through the afex package (See Tables A1.3-A1.12 in Appendix I for full model specifications and outputs). We included enclosure ID as a random factor in all models

to account for block effects. Because many of the response variables of interest in this study may be affected by an individual's body condition, we included this variable as a covariate in all models unless explicitly stated. We defined the body condition of each individual from the residuals of a least squares linear regression of body mass on SVL.

Data for each model were checked for violation of model assumptions, continuous response variables showing signs of heteroscedasticity were appropriately transformed and all model fits were checked for over dispersion using a Pearson's chi-square test. One Poisson distributed model (testing for the effect of treatment and phenotypic traits on male RS) was significantly over-dispersed; we corrected for overdispersion by including individual ID nested within enclosure as a random effect (see SI for details).

Home range and social pairings

We tested for treatment effects on HR size using LMMs with treatment, sex, SVL and a treatment by sex interaction term as fixed factors. To test whether treatment influenced the number of same-sex and opposite-sex conspecifics overlapping both male and female home ranges (hereafter intra- and inter-sex overlap, respectively) we conducted Poisson GLMMs with identical fixed factors.

To test whether or not treatment influenced the probability that males formed polygynous social groups we conducted binomial GLMMs with treatment and SVL as fixed factors. We also tested whether or not treatment influenced the probability that males were 'social' (partnered) or 'asocial' (not associated with a social partner) using a binomial GLMM taking treatment, inter-sex overlap and SVL as fixed factors.

Habitat quality

To verify that high and low quality habitat patches represented real differences in habitat quality, we tested for differences in body condition at recapture between individuals that had occupied high and low quality patches. Individuals were considered to have occupied high quality patches if >50% of their kernel home range occurred on high quality habitat. Body condition upon release was included as a covariate in these models to control for initial differences in body condition between individuals. For females we also included litter mass as a covariate to control for increases in body mass due to gestation.

Male and female reproductive success (RS)

We tested whether female RS differed between treatments with a Poisson GLMM taking treatment and SVL as fixed factors. We also tested whether or not treatment influenced the probability that a female became pregnant using a binomial GLMM with inter-sex overlap as an additional term. Due to the reduced sample size of females that were both assigned a social partner and successfully reproduced, we were unable to obtain adequate replication to include enclosure as a random effect when testing the influence of treatment on rates of extra-pair-paternity (EPP). Therefore this test was conducted using a binomial GLM with treatment, inter-sex overlap and SVL as fixed factors.

We tested whether males in monogamous and polygynous social groups had different RS with a Poisson GLMM taking social status (monogamous vs. polygynous), treatment and SVL as fixed factors. To test whether the influence of key phenotypic traits on male RS differed between treatments we conducted a Poisson GLMM taking treatment, SVL, head length as well as both two-way interactions with treatment as fixed effects. To generate standardized measures of head length that were not correlated with body size or body condition we performed a least squares linear regression of head length on SVL and used the residuals from that model as our head size measurement. Thus, a positive head length value indicates that the individual had a longer than average head relative to other individuals of the same body length.

We calculated the standardized variance in RS between males within an enclosure following Arnold and Wade (Arnold and Wade 1984). We also generated estimates of the strength of sexual selection on males using Bateman gradients. Bateman gradients characterize the strength of sexual selection on males and are general defined as the slope of the least squares regression of relative fertilization on relative mating success (Jones 2009). As we were unable to confidently calculate relative mating success for males (because copulations were observed too infrequently), Bateman gradients were calculated based on absolute values, derived simply from the least squares regression of male RS on male MS.

Ethics Statement

All work was carried out with approval from the Animal Ethics Committee at the University of Tasmania (Ethics Approval number A13390). Our use of toe clipping as a means of permanently marking animal subjects and tail tips as a means of acquiring

genetic material is based on peer reviewed literature evaluating the overall impact of marking methods in research involving lizards and was decided upon after thorough investigation of alternative methods (see SI for full justification of our methods).

Results

During two months of observations we recorded 2,788 positional observations and 42 behavioral interactions. From the initial 160 individuals, twenty (8 M, 12 F) could not be recovered at the end of the experiment and were assumed dead. These individuals were excluded from analyses of pregnancy, litter size and EPP but were included in analyses of HR size and overlap when their number of observations met minimum criteria. Of the 160 adults released, we had sufficient observational data to define kernels for 95% of males (n = 76) and 84% of females (n = 67).

Home ranges and male-female overlap

Treatment had a significant effect on HR size ($F_{1, 13.8} = 12.19$, $P = 0.003$), with both males and females maintaining larger home ranges in the aggregated treatment (Figure 2.1). Treatment also had a significant effect on inter-sex overlap for both males and females (Figure 2.2), with more male-female overlap occurring in the aggregated treatment (Females: $\chi^2 = 8.4$, $P = 0.004$; Males: $\chi^2 = 5.08$, $P = 0.024$). In contrast, treatment had no effect on the extent of same-sex overlap (Females: $\chi^2 = 1.87$, $P = 0.172$; Males: $\chi^2 = 3.79$, $P = 0.052$). However, female-female overlap was significantly less common than male-male overlap in both treatments ($\chi^2 = 7.82$, $P = 0.005$; Figure 2.3); 54% of females did not overlap with a single other female compared with 25% of males that did not overlap with another male. Body size (SVL) did not affect HR size or overlap for either sex (Females: HR size, $F_{1, 62.8} = 0.17$, $P = 0.682$; same-sex overlap, $\chi^2 = 0.67$, $P = 0.412$; inter-sex overlap, $\chi^2 = 2.73$, $P = 0.098$; Males: HR size, $F_{1, 71.9} = 1.91$, $P = 0.171$; same-sex overlap, $\chi^2 = 0.36$, $P = 0.548$, inter-sex overlap, $\chi^2 = 0.09$, $P = 0.762$).

After accounting for initial body condition, both males and females that occupied high quality habitat patches were in better body condition at the end of the experiment than those that occupied low quality habitat patches (males: $F_{1, 60.8} = 6.85$, $P = 0.011$; females: $F_{1, 61.3} = 4.85$, $P = 0.032$). Both sexes were also more likely to occupy high quality patches in the dispersed treatment (males: $\chi^2 = 6.3$, $P = 0.012$; females: $\chi^2 = 8.83$, $P = 0.003$).

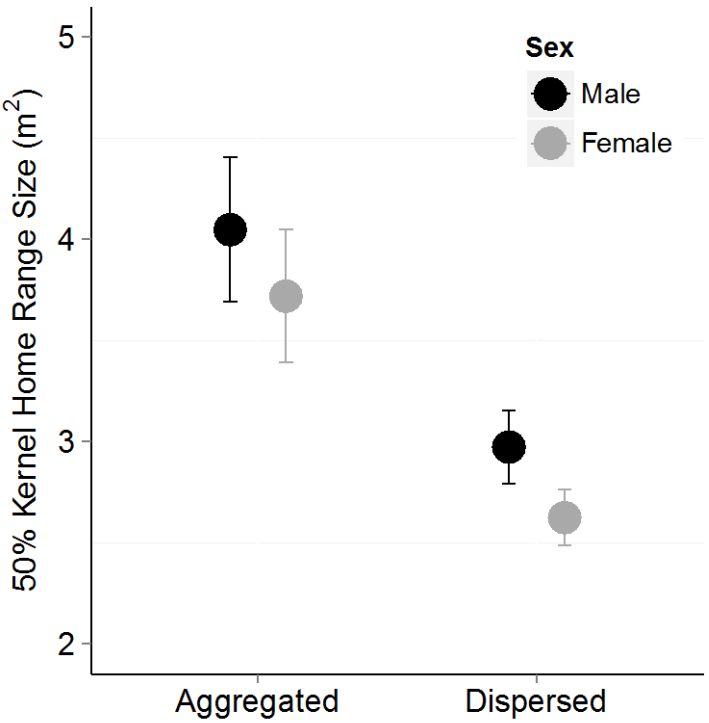

Figure 2.1. Mean (±SE) 50% kernel home range area (m^2) of male and female *Liopholis whitii* in the aggregated and dispersed treatments.

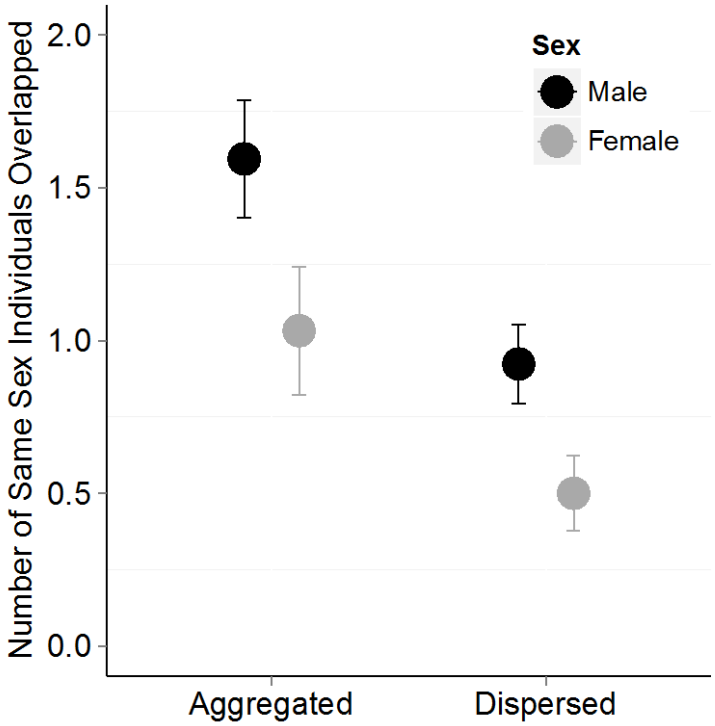

Figure 2.2. Mean (±SE) number of opposite sex individuals overlapped by males and females at the 50% kernel area in the aggregated and dispersed treatments.

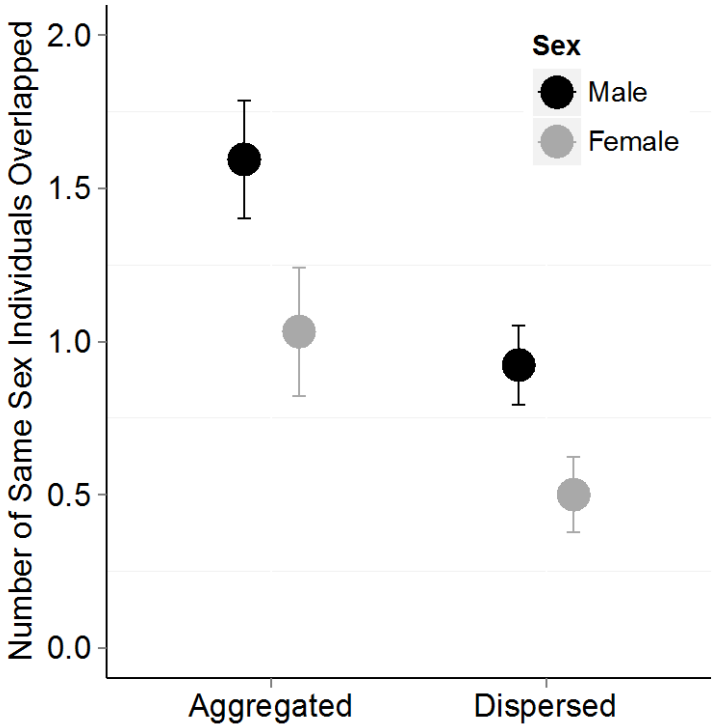

Figure 2.3. Mean (±SE) number of same sex individuals overlapped by males and females at the 50% kernel area in the aggregated and dispersed treatments.

Mating and social behaviour

Out of 76 males, 31 formed monogamous social groups, 13 formed polygynous social groups and 32 did not exhibit a stable social partner bond. Out of 67 females, 31 formed monogamous social groups, 27 were members of polygynous social groups and 9 did not exhibit a stable social partner bond. Treatment had a significant effect on the probability that males formed polygynous social groups, with more than three times as many polygynous groups occurring in the aggregated treatment ($\chi^2 = 5.17$, $P = 0.023$; Figure 2.4). This remained the case when conducting analyses on randomized data sets to validate the assignment of pairs (see methods) in all 10 tests, with at least three times as many polygynous social groups occurring in the aggregated treatment in each dummy data set ($\chi^2 > 4.04$, $P < 0.05$). Neither treatment nor SVL influenced the probability that males formed social groups (treatment: $\chi^2 = 1.41$, $P = 0.235$; SVL: $\chi^2 = 1.22$, $P = 0.268$).

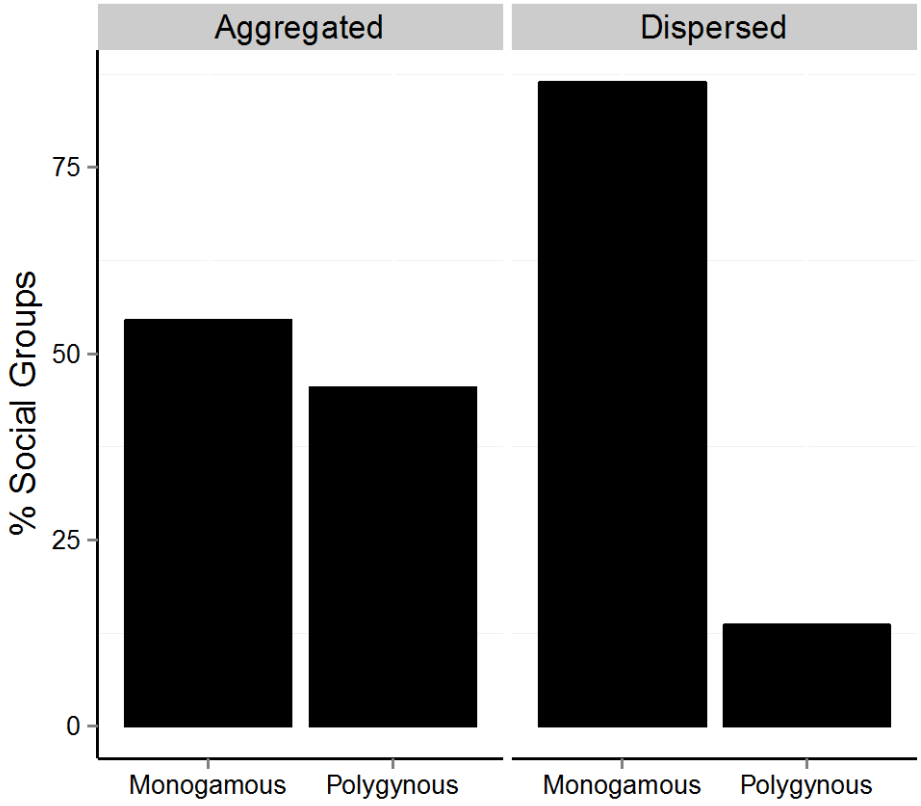

Figure 2.4. Proportion (% total) of social groups in each treatment that were monogamous (single female) versus polygynous (multiple females).

Reproductive success

Out of the 80 females released 38 (47.5%) produced a litter, giving a total of 82 offspring. Mean litter size was 2.16±0.12 g (range 1-3) and mean litter mass was 3.01±0.16 grams. The proportion of reproductive females and litter sizes are similar to that seen in natural populations (~60% of females per year; While et al. 2009). Body size (SVL) was the only significant predictor of whether females produced a litter (χ^2 = 9.35, P = 0.002); however, among reproducing females, neither SVL nor body condition influenced litter size (Table A1.11). Correspondingly, treatment had no effect on male reproductive success (RS), nor was there a difference in male RS between polygynous and monogamous social groups. Male RS was highly dependent on the number of genetic partners acquired (i.e., mating success (MS); χ^2 = 120.29, P < 0.001), indicating a strong positive Bateman gradient. However a significant treatment by MS interaction (χ^2 = 14.45, P < 0.001) revealed that the gradient was stronger for males in aggregated enclosures (Figure 2.5) than in dispersed enclosures. Specifically, a greater proportion

of sires from the aggregated treatment produced offspring with multiple females, resulting in a higher incidence of both genetic polygyny (50% vs. 18% of successful sires) and males that did not sire any offspring (68% vs. 58% of total males) in the aggregated treatment. This was also reflected in a greater standardized variance in RS amongst males within enclosures from the aggregated treatment (2.29 vs. 1.88), indicating greater potential for sexual selection under these conditions. Despite this, and although SVL was positively related to RS across treatments ($\chi^2 = 7.34$, $P = 0.007$), there were no significant interactions between treatment and any male phenotypic traits (Table A1.6).

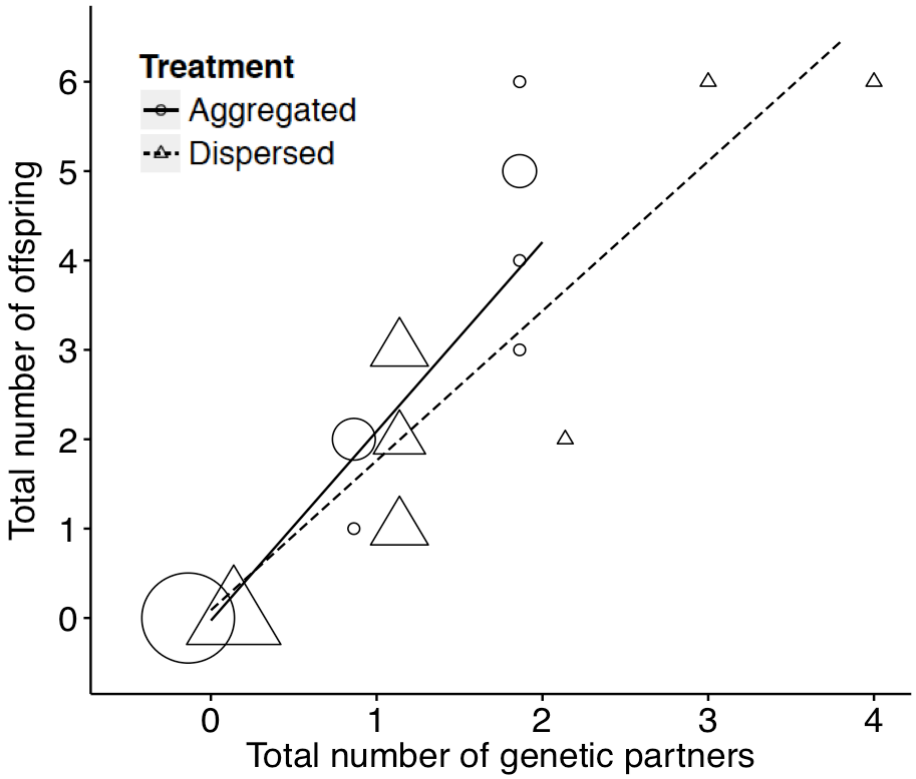

Figure 2.5. Linear regression showing the relationships between number of genetic partners acquired (MS) and total reproductive success (RS) for males in the aggregated and dispersed treatments. The slope of each line represents the Bateman gradient for each population. Symbol size represents sample size at each point (smallest symbol n= 1, largest symbol n = 27).

Treatment had no effect on rates of EPP ($\chi^2 < 0.01$, P=0.934); 64% of litters from the aggregated treatment and 59% from the dispersed treatment contained EP offspring. However the model indicated a marginally non-significant positive trend between inter-sex overlap and EPP ($\chi^2 = 3.73$, P=0.054). Multiple paternity was uncommon, occurring in only three of the 38 litters, all from females in the dispersed treatment. Thus, multiple paternity occurred in 15% of litters in the dispersed treatment and did not occur at all in the aggregated treatment. Due to this low occurrence we did not attempt any formal test of treatment effects on rates of multiple paternity

Discussion

Identifying the links between variation in habitat availability and variation in mating and social systems represents one of the fundamental challenges in behavioural ecology. Here we experimentally establish a causal relationship between variation in habitat availability and variation in social structure. We show that the distribution of high quality crevice sites plays an important role in mediating the social and mating system of a family living lizard. When crevice sites were aggregated into a central cluster, both males and females maintained larger home ranges, encompassing multiple high quality patches and forcing the remaining individuals to establish territories around peripheral low quality patches. In contrast, males and females in the dispersed treatment tended to form small discrete home ranges around a single high quality habitat patch. As a result, when habitat patches were aggregated, the clumped distribution of females facilitated a greater level of male-female overlap, a greater number of polygynous social groups, and increased potential for selection on sexual traits. In the dispersed treatment the majority of social groups consisted of a single male-female pair (e.g., social monogamy). These effects of resource distribution on social organisation are consistent with theoretical predictions (Emlen and Oring 1977), as well as empirical studies on a variety of taxa (Howard 1978; Wong et al. 2005; Martin and Martin 2007), including reptiles (e.g. M'Closkey et al. 1990; Hews 1993). Below we discuss the behavioral mechanisms that potentially mediate the observed effects and

the consequences of these results for our understanding of the maintenance and diversification of social organization in lizards.

The extent to which males are able to realize their potential for polygyny will depend on both the distribution of resources as well as on female territoriality and the tolerance of female-female overlap. When breeding females are aggressively territorial, female density will be reduced (for a given distribution of resources), limiting both opportunities and the economic feasibility of multiple mating (Shuster and Wade 2003; also see FitzGibbon 1997). In *L. whitii*, females exhibit similar levels of territorial aggression as males to both male and female conspecifics (Sinn et al. 2008; While et al. 2009). Interestingly, in our experiment, females maintained home ranges that were much more exclusive of one another than males, regardless of the distribution of resources. This intolerance most likely reduces the extent to which females formed dense overlapping aggregations like those observed in polygynous mating groups of other lizard species (e.g. Hews 1993; Jenssen and Nunez 1998) and may act to limit social polygyny in this system (e.g., social polygyny occurs only rarely in natural populations, ~20% of males) despite clear reproductive advantages of acquiring multiple mates for males (While et al. 2011). Nevertheless, we observed increased levels of social polygyny when crevice sites were aggregated. Since this was not due to greater female-female overlap in aggregated habitat, it may simply arise from reduced nearest neighbor distances between female territories, making it possible for dominant males to maintain exclusive social access to more than one female (see also Manzur and Fuentes 1979; M'Closkey et al. 1990). Therefore, pockets of high female density could be the principle reason that polygyny is a viable strategy for some male *L. whitii*. Such conditions have been shown to promote the evolution of social monogamy in mammals (Lukas and Clutton-Brock 2013; also see Komers and Brotherton 1997) and our study is consistent with the suggestion (Duffield and Bull 2002; O'Connor and Shine 2003; Michael and Cunningham 2010) that this has been important in the origin and evolution of family groups in *Egernia* as well.

Habitat distribution also had significant, albeit mixed, effects on male mating success. A higher proportion of sires from the aggregated treatment produced offspring with multiple females. This resulted in a higher relative rate of genetic polygyny, greater variance in male reproductive success and a steeper Bateman gradient in males from the aggregated treatment. This shows the importance of resource distribution for

selection on sexual traits in *L. whitii*. Interestingly, these results were independent of the difference between treatments in the frequency of monogamous *vs.* polygynous social groups. Indeed, males in polygynous social groups did not achieve higher reproductive success compared to males in monogamous social groups in either treatment. These results are in contrast with those from the wild for this species (While et al. 2011; also see Abell 1997; Lewis et al. 2000; Haenel et al. 2003 for other species). This is further supported by the fact that neither home range size nor inter-sex overlap were significant predictors of RS for males.

Although variance in male reproductive success differed between treatments, and male body size predicted reproductive success across treatments, we found no evidence for stronger selection on male phenotypic traits when crevice sites were aggregated. These results indicate a system of facultative and competitive polygyny in which large males achieve the greatest reproductive success regardless of the distribution of resources. This is in contrast to natural population studies, which indicate no effect of body size on male RS in *L. whitii* (McEvoy et al. 2013), and could reflect an artifact of elevated competition imposed by experimental conditions (Keogh et al. 2013). Indeed, there is no strong evidence of sexual size dimorphism among other species in the *Egernia* group (Chapple 2003). Sexual size dimorphism has been linked to male territoriality and social polygyny in lizards (Stamps 1983; Olsson and Madsen 1998). Therefore, female intolerance may also play a role in constraining sexual selection on male body size in *L. whitii* by undermining the advantages of large males in maintaining polygynous social groups, especially in areas with low population density.

Rates of EPP did not vary across treatments or social group types, but were considerably higher than those reported from natural populations (While et al. 2011). One explanation for this discrepancy is that rates of EPP were simply a product of abnormally high male-female encounter rates (see Uller and Olsson 2008 for a review of this topic). Alternatively, the high levels of EPP could be the outcome of scramble competition for mating when adults were first introduced into the enclosures. Specifically, if some males were successful in securing opportunistic matings with females other than their eventual social partner(s) before territories were fully established, this would necessarily inflate our estimates of EPP. Whatever the processes that explain rates of EPP found in this study, the general association between inter-sex

overlap and EPP demonstrates that variables influencing the spatial association of potential mates will be important in determining the mating behavior of female *L. whitii*.

In summary, we found strong effects of the distribution of a key resource on various social and mating strategies in a social lizard. Our results are largely consistent with theoretical predictions (*sensu* Emlen and Oring 1977), yet also support recent research demonstrating the role of female territoriality in mediating social spacing and the environmental potential for polygyny. These findings add weight to hypotheses advocating the importance of habitat structure in the processes that have led to the emergence and maintenance of sociality across the *Egernia* group (Duffield and Bull 2002; O'Connor and Shine 2003; Chapple and Keogh 2006; Michael and Cunningham 2010) and provide clear, testable hypotheses for future research. Specifically, where resource patches are small and uniformly distributed across the landscape we expect small social groups centered around monogamous pair bonds to form. Where resources are heterogeneously distributed in clumped patches this should promote opportunities for social polygyny, potentially facilitating the accommodation of female-female tolerance and the emergence of larger multi-female social groups. When resources are abundant and population densities are high, male territoriality may become uneconomical (Grant 1993; Maher and Lott 2000), promoting the formation of multi-female, multi-male groups, such as those observed in E. *stokesii* (Gardner et al. 2001). A comparative approach, studying a range of populations and species, will be necessary to rigorously test these predictions.

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Data Accessibility Statement

Analyses reported in this article can be reproduced using the data provided by Halliwell (2016).

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Chapter Three

Habitat Structure Influences Parent-Offspring Association in a Social Lizard

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Abstract

Parental care emerges as a result of an increase in the extent of interaction between parents and their offspring. These interactions can provide the foundation for the evolution of a range of complex parental behaviors. Therefore, fundamental to understanding the evolution of parental care is an understanding of the factors that promote this initial increase in parent-offspring association. Here, we used large outdoor enclosures to test how the spatial structure of high-quality habitat affects the occurrence of parent-offspring associations in a social lizard (*Liopholis whitii*). We found that the extent of parent-offspring association was higher when high-quality habitat was aggregated relative to when it was dispersed. Associating with parents had significant benefits for offspring growth and body condition but there were no concomitant effects on offspring survival. We did not find costs of parent-offspring association for parents in terms of increased harassment and loss of body condition. We discuss a number of potential mechanisms underlying these results. Regardless of mechanisms, our results overall suggest that habitat structure may shape the extent of parent-offspring association in *L. whitti*, and that highly aggregated habitats may set the stage for the diversification of more complex forms of care observed across closely related species.

Introduction

The evolution of parental care is associated with an increase in the level of social interactions between parents and offspring (Clutton-Brock, 1991; Smiseth et al., 2012). The result is a range of complex and diverse parental behaviors (e.g., parental provisioning, care after nutritional dependence) which can have profound effects on offspring development and fitness (Clutton-Brock, 1991; Uller, 2012; Klug and Bonsall, 2014). These characteristics also make parental care a key point in the adaptive radiation of kin relationships and sociality (Queller, 1994; Field and Brace, 2004). Thus, there is enormous interest in understanding the factors responsible for the initial emergence and subsequent diversification of parental care.

The majority of research on the evolution of parental care has focussed on identifying the benefits of parental investment for offspring fitness (reviewed in Clutton-Brock, 1991; Royle et al., 2012). Such benefits may be necessary for parental care to be selected; however, the emergence of parental care will be facilitated, first and foremost, when parents regularly encounter and associate with their offspring (Lion and van Baalen, 2007). Therefore, conditions that promote increased levels of association between parents and their offspring will be central to facilitating the early emergence of parental care (Wilson 1975; Lion and van Baalen, 2007; Davis et al., 2011; Klug et al., 2012). Habitat availability and quality are particularly important in this context. For example, limited availability of suitable habitat can encourage offspring to delay dispersal and remain within the parental home range (Hatchwell and Komdeur, 2000; Covas and Griesser, 2007). If this carries little or no cost to the parents, they may tolerate offspring, resulting in an increased level of parent-offspring association. Where these environmental conditions are recurrent, parent-offspring associations can create a novel selective environment from which more complex forms of parental care, such as parental provisioning, can evolve (e.g., Wong et al., 2013). Indeed, theoretical models have shown that once this initial increase in parent-offspring association emerges parental care can rapidly diversify and increase in complexity (e.g., Gardner and Smiseth, 2011).

Species in which parent-offspring associations are facultative or temporary, such as those exhibited by some insects, fish, amphibians and lizards, provide excellent opportunities for establishing the conditions that promote the early evolution of

parental care (Kölliker, 2007; Falk et al., 2014). In lizards, post hatching parent-offspring associations have been demonstrated in at least 60 species (Somma, 2003; While et al. 2014). In most cases these associations are characterized by semi-independent offspring remaining within the parental home range (While et al., 2014). While this level of parent-offspring association is considerably simpler than in many other vertebrates, it is characteristic of what we would expect in the early stages of the evolution of postnatal parental care. Thus, these taxa provide an opportunity to study how selection on more complex forms of parental care initially arise, by examining the factors that influence increased parent-offspring association and the costs and benefits of this association for both parties.

Here we conduct an experimental test of how habitat structure influences parent-offspring associations and the consequences of this for offspring growth and survival in a social lizard species, *Liopholis whitii*. *Liopholis whitii* lives in family groups characterized by stable (often life-long) male-female pair bonds and prolonged parent-offspring associations (Chapple and Keogh 2005; 2006; While et al., 2009a). These prolonged associations involve offspring delaying dispersal and parents tolerating offspring within their core home ranges, sometimes for up to several years. This has two potential benefits to offspring. First, offspring that associate with their parents may gain access to parental resources (i.e., food within a parent's habitat) and hence benefit in terms of increased growth and/or condition (O'Connor and Shine, 2004; but see Langkilde et al., 2007). Second, offspring may gain survival benefits through protection from infanticide (Sinn et al., 2008). Tolerance of offspring within the home range may, however, also have costs to adults. For example, parental body condition may be reduced through sharing resources with their offspring. Parents may also suffer injury and/or reduced body condition through increased harassment from hungry conspecifics.

We manipulated habitat structure by manipulating the distribution of available crevices sites. Rock crevice and burrow sites are a key component of *L. whitii's* ecology and it has been suggested that the structure and availability of these sites is fundamental in determining the extent of parent-offspring associations in *L. whitii* and related species (Duffield and Bull, 2002; While et al., 2009a). We created two experimental treatments which differed in the spatial association of crevice sites, a dispersed crevice site treatment whereby available crevice sites were dispersed evenly

across the environment and an aggregated crevice site treatment, where available crevice sites were clumped together in a central location. We predicted that the incidence of parent-offspring associations would be lower when suitable crevice sites were clumped, as clumping of high quality habitats should lead to more frequent agonistic encounters over access to high quality habitats between adult lizards from different pairs and thus result in higher costs (and hence a lower incidence) of parents tolerating offspring within their home range and defending these offspring from conspecific aggression.

Methods

Study species

Liopholis whitii is a medium sized (75-100 mm snout-vent length (SVL)) viviparous skink that occurs throughout south-eastern Australia, including Tasmania (Chapple, 2003; Wilson and Swan, 2003). It occupies a broad range of habitats (including coastal heaths, grasslands, woodlands and dry sclerophyll forests) and altitudes (0-1600 m) (Cogger, 2003; Chapple, 2003; Wilson and Swan, 2003). Typically, *L. whitii* are closely associated with complex burrow systems under/around rocks and shrubs (Chapple, 2003; Wilson and Swan, 2003) where they typically focus their basking and foraging activities (Greer, 1989). Morphological and life history traits vary geographically in *L. whitii* (Chapple, 2005). Tasmanian populations are sexually monomorphic, mature at approximately three years and have a lifespan of 9-10 years (While et al., 2009b). Reproduction occurs annually, with breeding occurring in the austral spring (September-October) and gestation lasting 3-4 months (While et al., 2007). Parturition occurs in the austral summer (January-February) with litters comprising one to four offspring (most frequently two) born asynchronously, usually over several days (While et al., 2007).

Experimental Protocol

We caught 160 *L. whitii* (80 males, 80 females) sourced from populations on the east coast of Tasmania (approximately 42°57’ S, 147°88’ E) at the start of the breeding season (September 2013). Once captured, animals were transported in cool, damp cloth

bags back to the University of Tasmania (approximately an hour drive from the populations). At the University, lizards were weighed (± 1 mg), measured for SVL and total length (± 0.5 mm), indications of previous tail loss recorded, and gender determined via eversion of hemipenes. Each lizard was uniquely toe-clipped to enable individual identification. Lizards were then housed individually in plastic terraria (30 x 60 x 40 cm) kept under a 25 W basking light set to an 8:16 hour light/dark cycle with overhead lights set on a 10:14 hour light/dark cycle. Each terrarium had a basking rock underneath the basking light, with a wooden shelter at the opposite end of the shelter. Lizards were provided with water and food (*Tenebrio* larvae and fruit puree mixed with protein powder) *ad libitum*. Lizards were then moved to our large enclosure facilities at the University of Tasmania's Cambridge Farm facility (16 enclosures, each measuring 8 x 8 m) and assigned to one of two experimental treatments (eight enclosures per experimental treatment).

The enclosures consisted of eight replicates for each of two treatments: 1; a dispersed crevice site treatment, and 2; an aggregated crevice site treatment. The crevice sites within the enclosures were constructed from either a wooden pallet, six hollow concrete bricks (Besser blocks) and sand (representing high quality habitat) or two Besser blocks only (representing low quality habitat). Each treatment had five of these high quality crevice sites and four low quality crevice sites, but the arrangement of crevice sites between treatments differed, with the high quality crevice sites spread apart in the dispersed treatment and aggregated together in the center of enclosure in the aggregated treatment (Figures 3.1, A2.1). Enclosure treatments were paired, with the order of treatments randomized within each pair. All enclosures were covered by bird netting to prevent predation by birds. While this removes potential sources of mortality for both adult and offspring it is unlikely to influence the costs and benefits of parent-offspring association themselves. Parent-offspring associations are unlikely to function in a predator protection context, given that the majority of predators are significantly larger than an adult *L.whitii* (e.g. include feral cats, snakes and large birds such as kookaburras and ravens). Indeed, the main source of mortality for offspring and harassment for parents is conspecifics (Lanham and Bull, 2000; O'Connor and Shine 2006). The enclosures were stocked with water containers and live insects throughout the duration of the experiment, with these resources distributed evenly throughout the enclosures.

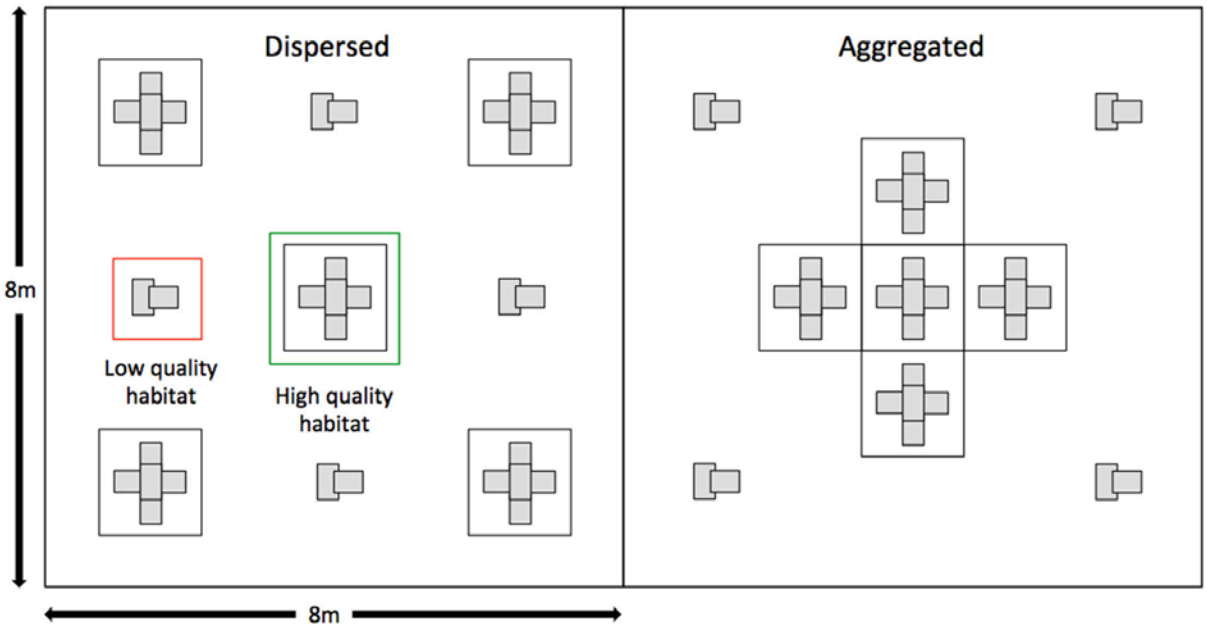

Figure 3.1. Diagrammatic representation of the two treatments used in the experiment. On the left is the dispersed habitat treatment and on the right is the aggregated treatment. Areas representing high quality and low quality habitat indicated.

Lizards were introduced into enclosures in October 2013. Five females and five males were randomly assigned to each enclosure. This represented a similar, albeit slightly higher, density to that found in natural populations (G. M. While pers. observation). This also resulted in a match between the number of male/female pairs and number of high quality crevice sites in each enclosure. Hence, the treatments differed only in the layout of the high quality crevice sites, which were expected to be preferentially occupied by male/female pairs. Lizards were semi-permanently marked with numbered cloth tape (Tesa, Hamburg, Germany) to enable identification through observation of individuals. From October to December 2013, the lizards were observed up to twice daily, once in the early morning and once in the afternoon, by one experimenter (BH). These time periods were chosen to correspond with the time when lizards are most active and do the majority of their basking before seeking shelter in the middle of the day. The order of observations was rotated so that the starting enclosure differed each observation session, while the order or enclosures observed was consistent. Due to the weather dependence of lizard activity it was not always possible to record observations at set times and in these instances observations were taken opportunistically during the day whenever the weather permitted activity. During

observation session, data were collected on the locations of lizards in each enclosure and additional data were taken on any observed interactions between lizards (such as fights or copulations). Observations were made at least one meter from each enclosure to avoid disturbing lizards, and an observation session ended when all enclosures had been thoroughly observed (typically taking 10 minutes per enclosure) or when the lizards' activity period finished (i.e., when the weather became too hot or cold). If two observation sessions were taken on the same the day, they were taken at least four hours apart to reduce spatial autocorrelation of individual locations. In total we collected 2,874 observations.

At the start of January 2014 (i.e., at the end of gestation) individuals were captured from the enclosures, brought back to the University, and housed as described above. Upon return to the laboratory individuals were measured for the same traits taken at the start of the breeding season (see above). Female terraria were checked daily for the birth of offspring. Upon birth, the date of birth was recorded and offspring weight (± 1 mg (SE)), SVL (± 0.5 mm) and total length (± 0.5 mm) were measured. Each juvenile was then given a unique toe-clip for identification purposes. In total 67 females were recaptured in January, 37 of which gave birth. This represents 55% of the female population reproducing, which is equivalent to that observed in the natural population where only 68% of females give birth in a given year (While et al. 2009; see also Chapple 2003 for the consistency of this pattern across Egernia species). Before release, offspring were marked with a colored bee tag attached with non-toxic glue (Pender Beekeeping Supplies), with five different colors corresponding to a particular mother (Figure A2.2). Position of the tag along the offspring's back identified which member of a litter it was (on the neck = 1st born offspring, on middle of the back = 2nd born offspring, on the pelvis = 3rd born offspring; no litters contained more than three offspring).

All individuals were then re-released into the enclosures from early to late February 2014. All individuals were released at the crevice site within the enclosure that they were most frequently observed (based on the 2,874 observational data points; see above). Offspring were released with their mother at their mother's main crevice site. Daily observations were then conducted across both treatments by two experimenters (TBJ and EB) following the same protocols described for the October-

December 2013 period. This resulted in a total of 4,235 independent observations for all individuals combined over 85 observational sessions.

Lizards were recaptured from late April to early May 2014, and brought back to the University. On average, parents and offspring were in the enclosures for 58.75 ± 0.52 days. The adults were measured for weight (± 1 mg), SVL (± 0.5 mm), total length (± 0.5 mm), toe and tail loss. The juveniles were measured for weight (± 1 mg), SVL (± 0.5 mm), total length (± 0.5 mm) and tail loss. All individuals were then released back into the natural populations from which they came.

Parentage Assignment

All individuals included in the study were genotyped for six microsatellite loci (EST1, EST2, EST4, EST12: Gardner et al., 1999; TruL12, TruL28: Gardner et al., 2008) using standard molecular techniques with DNA extracted from tail tip samples (see While et al., 2009a, b for further details). Paternity was assigned using the computer program CERVUS 3.0 (Marshall et al., 1998) using the following simulation parameters: 10,000 cycles, 95 % of candidate parents sampled, 85 % loci typed and a genotyping error rate of 1 % (calculated in CERVUS from our data). The one known parent option was used with all adult males released into the same enclosure as the mother included as possible fathers. Paternity was assigned to the male with the highest male-female-offspring trio LOD score and the lowest number of mismatches (0 or 1) (e.g., Foerster and Kempenaers, 2004; Chapple and Keogh, 2005). Because there were only five possible fathers for any offspring within an enclosure, paternity could be assigned with high confidence in the majority (>90 %) of cases. Seven out of 76 offspring could not be confidently assigned paternity (had ≥2 loci mismatches) and were thus excluded from analyses of father-offspring association.

Home range analysis and assignment of parent-offspring associations

Parent home ranges were constructed using the program ArcView3.3 (ESRI) using a fixed kernel analysis with a least squares cross-validation smoothing parameter (Powell, 2000). Core home range was calculated using 50 % isopleths. For *L. whitii* and related species, this area represents an individual's permanent shelter site from which it basks, feeds and undertakes the majority of its social behavior (e.g., While et al., 2009a). Adults with less than eight observations were excluded from the analysis (n =

20) as home ranges could not be constructed for these individuals. The low number of average sightings of juveniles relative to adults (juveniles = 8 ± 1, adults = 25 ± 1) prevented the assignment of presence or absence of parent-offspring association based on parent-offspring home range overlap. Instead, based on long-term monitoring of a wild population for which home range overlap is available (While et al. 2009a,b) we defined a parent-offspring association when juveniles had 50% or more of their observations within their parent's core home range area (see also While et al., 2009a, b). The average percentage of observations for offspring assigned as being associated with their parents was 73 ± 5 % and 72 ± 9 % for mothers and fathers respectively compared to 8 ± 2 % and 3 ± 1 % for offspring who were not associated with their parents.

Data analyses

Data were analyzed using General and Generalized Linear Mixed Models fitted using maximum likelihood implemented in R version 3.0.2 (R development core team 2014), using either the 'glmer' (for binary response variables) or 'lmer' function (for continuous response variables) under the 'lme4' package (Bates et al., 2012). All models used the Laplace approximation to estimate model parameters, as it is considered a more accurate technique than the simpler pseudo quasi-likelihood estimation method (Bolker et al., 2009). Models regarding offspring traits included parental ID as a random factor to account for non-independence arising from litters containing multiple offspring. All models also included enclosure as a random factor to account for differences between enclosures. Because of limited sample size we ran main effects models only and models for maternal and paternal parental-offspring association were run separately. The low incidence of bi-parental parental-offspring association (only 2 cases total) precluded its analysis.

All fixed effects were tested with Wald's χ^2 and type III *F* tests (Kenward-Rogers approximation for *F* tests) obtained with the 'car' package (Fox et al., 2014). All models were checked for violation of assumptions. All results are reported as means, with standard errors as the measure of variability.

Parent-offspring association

The effect of habitat structure on the extent of parent-offspring association was analyzed by examining the proportion of parents whose offspring remained within their home range, using a Generalized Linear Mixed Model with the binomial family specified. These models included treatment (clumped vs. aggregated treatment) as a fixed factor, parental body condition as a covariate, and enclosure as a random factor. Body condition (as an indicator of an individual's energy stores relative to structural components of the body) was measured by taking mass divided by SVL. This has been suggested as a reliable index of body condition (Green, 2001; Labocha et al., 2014). Analyses of body condition excluded individuals who had lost tails (as tail loss affects mass and therefore estimates of body condition). We then examined the relative occurrence of parent-offspring associations on high versus low quality habitats between treatments, and the extent to which adults and offspring occupied high versus low quality habitats between treatments. We assigned individuals as occupying either high or low quality habitat based on the location of their home ranges (for adults) or where 50 % or more of their observations occurred (for offspring). Individuals whose home range or majority of observations occurred primarily on grass areas (i.e., neither pallets nor Besser blocks) were excluded from analysis. These analyses were run using Generalized Linear Mixed Model's with treatment as a fixed factor and enclosure as a random factor.

Consequences of parent-offspring association for offspring

Benefits of parent offspring association for offspring were analyzed in terms of skeletal growth, body condition and survival. Change in SVL between the start and end of the experiment was used to assess offspring skeletal growth (SVL is a common measure of growth for reptiles – e.g., Shine and Charnov, 1992). Analysis of growth used a General Linear Mixed Model with treatment (i.e., aggregated vs. dispersed habitat treatments) and mother-offspring association as fixed factors, the number of days spent in an enclosure as a covariate, and maternal and enclosure ID as random effects. The difference in offspring body condition between treatments at the end of the experiment was analyzed using a Generalized Linear Mixed Model with treatment and mother-offspring association as fixed factors, initial offspring body condition and the number of days spent in an enclosure as covariates, and maternal and enclosure ID as random

effects. As there were only 3 cases where offspring associated with their father and we had corresponding measurements for offspring growth and body condition, we ran the above models for mother-offspring association only. Finally, we analyzed differences in offspring survival by running a Generalized Linear Mixed Model on the survival status of offspring (recaptured = survived, not recaptured = dead) at the end of the experiment. These models had mother-offspring association (yes or no), father-offspring association (yes or no) and treatment as fixed factors and parental and enclosure ID as random effects.

Consequences of parent-offspring association for parents

Increased parent-offspring association may have a number of fitness costs for parents in terms of decreased body condition associated with sharing resources with offspring and increased harassment from other lizards. To test how marks of harassment varied between parents who associated with their own offspring and those who did not and between the two treatments, we used both tail and toe loss as a proxy (loss of tails and toes are key indicators of intraspecific competition in lizards, especially where predation has been eliminated: Norris, 1953; Tinkle, 1967; Vitt et al., 1974). Tail and toe loss were entered as a binary response variable in four separate main effects models (one for each sex and each trait), with parent-offspring association and treatment as fixed factors. Including enclosure ID as a random factor resulted in poor model convergence and was excluded from these models. To test consequences of parent-offspring associations for adult body condition, we ran a General Linear Mixed Model with parent body condition at the end of the experiment as a response variable, parent-offspring association and treatment as fixed factors, initial parental body condition as a covariate, and enclosure I.D. as a random factor.

Results

Seventy six offspring were born in the laboratory to 37 mothers (average brood size = 2.05 ± 0.13) and released with their parents into the large outdoor enclosures. Thirty nine of the 76 offspring released into the enclosures were recaptured at the conclusion of the observation sessions in April/May, representing an overall survival of 53 %. Survival of adults from release at the start of February until April/May was high, at 95

%. Average adult home range size during this period was the same between treatments (aggregated = 7.81 ± 0.72m^2, dispersed = 6.42 ± 1.05m^2; $F_{1, 13.58} = 1.20$, p = 0.29) and between males and females (males = 6.34 ± 0.51m^2, females = 7.89 ± 1.20m^2; $F_{1, 122.78} = 1.48$, p = 0.23). The extent to which low versus high quality crevice sites were occupied by adults differed significantly between treatments ($\chi^2 = 21.44$, $p < 0.01$). In the aggregated treatment 14 of the 39 adults who occupied crevice sites occupied high quality sites (36 %) compared to 46 of the 54 adults (85 %) in the dispersed treatment. For offspring in the aggregated treatment 6 of the 13 (46 %) offspring who occupied crevice sites occupied high quality sites compared to 5 out of the 10 offspring (50 %) in the dispersed treatment ($\chi^2 = 1.00$, p = 0.32). The remaining adults and offspring established themselves away from the crevice sites in grass patches or the perimeter of the enclosure.

Parent-offspring association

Of the 37 females who produced offspring, there was sufficient observational data to establish the extent of mother-offspring association for 34 mothers. Overall, 12 out of these 34 mothers (35 %) associated with their offspring. The extent of mother-offspring association differed significantly between treatments (Table 3.1). Specifically, 9 out of 15 mothers (60 %) associated with their offspring in the aggregated treatment compared to 3 out of 19 (16 %) in the dispersed treatment. Of the 28 males who sired offspring, there was sufficient observational data to establish the extent of father-offspring association for 25 fathers. Overall, 7 of these 25 fathers (28 %) associated with their offspring; 5 out of 12 fathers (43 %) in the aggregated treatment and 2 out of 13 (15 %) in the dispersed treatment. This difference failed to reach statistical significance (Table 3.1). There was no effect of a mother or father's initial body condition on whether or not they associated with their offspring (Table 3.1). An analysis at the offspring level produced qualitatively similar results, with 35% and 19% of offspring in the aggregated treatment associating with their mother and father respectively, compared to 9% and 10% in the dispersed treatment. However, these differences were not statistically significant (Mother: $\chi^2 = 2.32$, p = 0.13; Father: $\chi^2 = 0.095$, p = 0.33). The ratio of parent-offspring associations formed on low versus high quality crevice sites was higher in the aggregated compared to the dispersed treatment. Only five out of 14 parent-offspring associations (36 %) in the aggregated treatment occurred on high

quality crevice sites, compared to 4 out of 5 (80 %) in the dispersed treatment. This difference, however, was not significant ($\chi^2 = 0.95$, $p = 0.33$).

Consequences of parent-offspring association for offspring

Sixty nine percent (9/13) of offspring that associated with their mother survived, compared to 62 % (25/40) offspring that did not ($\chi 2 = 0.06$, $p = 0.81$). These results were mirrored in the data collected on paternal-offspring association ($\chi 2 = 1.85$, $p = 0.17$). Specifically, 3 out of 7 (43 %) offspring that associated with their father survived, and 25 out of 40 (62 %) offspring that did not associate with their father survived. Offspring survival did not differ between treatments (aggregated treatment = 57 % (20/35), dispersed treatment = 43% (19/41); $\chi^2 = 1.03$, $p = 0.31$).

Table 3.1. Output from models testing for differences in parent-offspring association in *Liopholis whitii* between treatments and in relation to parental condition.

Factor	Mother-Offspring		Father-Offspring	
	χ2	***P***	***χ2***	***P***
Treatment	4.58	**0.03**	3.57	0.06
Parental Condition	0.29	0.58	1.28	0.26

Mother-offspring association had a significant effect on offspring growth and body condition (Table 3.2). Offspring that were associated with their mother had increased growth and were in better body condition at the end of the experiment relative to those who were not (Figure 3.2A and Figure 3.2B). There was no significant effect of treatment on either offspring SVL growth or change in body condition (Table 3.2).

Consequences of parent-offspring association for parents

We found no costs of increased parent-offspring association for mothers or fathers in the form of harassment suffered from conspecifics (e.g., frequency of tail and toe loss did not differ between treatments for mothers or fathers; Table 3.3). There was no difference in mother or father body condition at the end of the experiment between those parents who did and did not associate with their offspring nor were there any differences between treatments (Table 3.3).

Table 3.2. Output from models testing for effect of treatment and parent-offspring association on offspring growth and condition in *Liopholis whitii*. Statistically significant P-values are in bold.

Factor	Offspring SVL Growth		Offspring Condition	
	F	***P***	***F***	***P***
Treatment	$F_{1,7.42} = 0.01$	0.91	$F_{1,4.81} = 0.33$	0.59
Maternal Association	$F_{1,24.85} = 5.51$	**0.03**	$F_{1,12.14} = 9.16$	**0.01**
Days in Enclosure	$F_{1,24.17} = 24.17$	**0.04**	$F_{1,14.83} = 5.41$	**0.03**
Offspring Condition at Release			$F_{1, 12.62} = 13.28$	**<0.01**

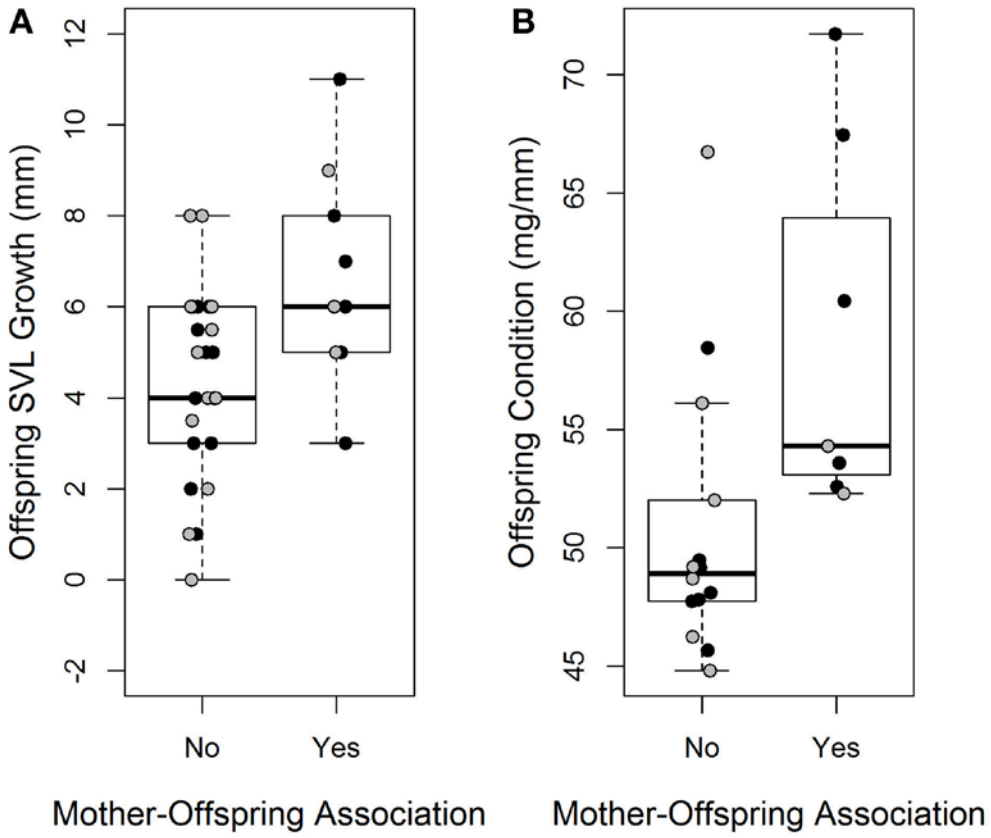

Figure 3.2. Difference in offspring SVL growth (mm) (A) and offspring body condition (mg/mm) (B) between offspring who associated with their mother and those who did not. Black data points indicate offspring from the clumped treatment, grey data points indicate offspring from the dispersed treatment

Table 3.3. Output from models testing for effect of treatment and parent-offspring association on parental condition and harassment in *Liopholis whitii*. Statistically significant P-values are shown in bold.

Factor	Mother Toe Loss		Father Toe Loss		Mother Tail Loss		Father Tail Loss		Mother Condition		Father Condition	
	χ2	***P***	***χ2***	***P***	***χ2***	***P***	***χ2***	***P***	***F***	***P***	***F***	***P***
Treatment	0.02	0.89	1.62	0.20	0.23	0.63	0.05	0.82	$F_{1,15.02} = 1.77$	0.20	$F_{1,6.53} = 0.84$	0.39
Parental Association	0.43	0.51	1.62	0.20	0.82	0.37	0.92	0.34	$F_{1,19.73} = 3.58$	0.07	$F_{1,11.98} = 0.81$	0.38
Days in Enclosure	4.26	**0.04**	0.45	0.50	1.31	0.25	1.37	0.24	$F_{1,18.82} = 2.17$	0.16	$F_{1,9.97} = 0.02$	0.88
Release Condition									$F_{1,13.96} = 7.02$	**0.02**	$F_{1,10.61} = 20.41$	**<0.01**

Discussion

Testing how the structure of the environment influences associations between offspring and their parents is fundamental to our understanding of the origins of parental care (Gardner and Smiseth, 2011; Klug et al., 2012). Here we show that approximately a third of males and females associate with their offspring following birth. This level of parent-offspring association is in accordance with what is observed in the wild, where the number of parents associating with offspring can vary from between 10 – 70% (While et al. 2009b; Botterill-James et al. unpublished data). We further show that the structure of high quality habitat significantly influenced the extent of parent offspring association. This increased parent-offspring association has benefits for offspring growth and body condition, but does not appear to carry a substantial cost for parents. Below, we discuss our results in the context of findings in other species, the mechanisms which may underlie the observed effects of habitat structure on parent-offspring associations, and discuss the broader implications of these findings for the evolutionary origins of more complex forms of parental care.

Habitat structure and availability is an important ecological variable in *Liopholis whitii*, which has been suggested to influence the social complexity of this and other species of *Egernia*. (Duffield and Bull, 2002; Chapple, 2003; O'Connor and Shine, 2003; While et al., 2009a). Here we experimentally demonstrate that the spatial aggregation of high-quality crevice sites promote parent-offspring association. Specifically, both mothers and fathers were more likely to associate with their offspring when high quality habitat was aggregated compared to when it was dispersed, although that latter result failed to reach statistical significance. These results are consistent with the suggestion that the availability and structure of habitat are key to facilitating the evolution of postnatal parental care by increasing habitat sharing between closely related individuals (Wilson, 1975; Lion and van Baalen 2007). This is believed to be fundamental to the formation of family groups across the *Egernia* (Duffield and Bull, 2002; Chapple, 2003; O'Connor and Shine, 2003; While et al., 2009a), but current empirical evidence for this hypothesis is mixed. For example, manipulation of shelter availability in *E. striolata* altered adult pair bonding, with more pairs forming when shelter availability was low (Lancaster et al., 2010), whereas Gardner et al. (2007) found no effect of crevice site abundance on social group structure in *Egernia stokesii*.

Despite a general effect of habitat structure on parent-offspring association the direction of this effect requires some explanation. Specifically, there was a greater level of parent-offspring association when high-quality habitat was aggregated compared to when it was dispersed. This is perhaps counter-intuitive; it might be expected that there would be strong costs to parents from associating with offspring in the aggregated treatment, due to increased harassment from conspecifics relative to the dispersed treatment. However, we found little evidence that parental-offspring association carries costs to either parent. The analysis looking at where adults and offspring settled within enclosures suggests an alternative explanation. Adult, but not offspring, occupation of low versus high quality habitats differed between treatments; more adults were present on low quality habitats in the aggregated treatment, probably as a result of competitive exclusion from home ranges of dominant individuals. There were also more parent-offspring associations formed on low versus high quality habitats in the aggregated treatment (although the low statistical power limited the confirmation that this deviated from the null expectation of no difference between habitats). The tight spacing of crevice sites in the aggregated treatment may therefore have facilitated their monopolization by a small proportion of adults while the majority of (more subordinate) adults were forced into the lower quality areas. This would then increase habitat saturation and reduce the overall availability of crevice sites (both of high and low quality) facilitating greater overlap of habitat use between these adults and their offspring, with this overlap then maintained by no/low costs of parent-offspring association for adults. Therefore, enhanced parent-offspring association may be a result of some adults being restricted to low quality habitats where the majority of offspring are residing as opposed to any benefits of delayed dispersal to offspring *per se*. This supports natural population data on *E. saxatilis*, where habitats occupied by solitary versus parentally-associated offspring were similar when measured across a range of habitat quality indicators (Langkilde et al., 2007). Further tests are required to confirm whether the proposed explanation of habitat monopolization (and forced habitat sharing between ousted parents and their offspring) is the mechanism responsible for the observed pattern of parent-offspring association. This could be achieved by directly manipulating habitat density rather than structure or by altering dominance-subordination hierarchies within enclosures (similar manipulations have been performed, for example, to examine the evolution of sociality in coral fish – Buston,

2004). Additionally, this mechanism could be investigated by observing natural populations and determining the frequency of parent-offspring associations across environments that differ in density (and hence availability) of suitable habitats, and determining whether less dominant individuals (assessed by behavioral interactions or their location on lower quality crevice sites) more often associate with their offspring.

There were clear benefits for offspring that associated with their mother. Specifically, offspring who resided within their mother's home range grew significantly more and were in significantly better body condition at the end of the experiment than offspring who did not. There are at least two mechanisms that could lead to these benefits. First, parental protection from conspecific harassment may allow offspring to spend more time foraging freely. Such an effect has been demonstrated in the laboratory for the related *Egernia saxatilis* (O'Connor and Shine, 2004). Second, offspring growth could simply result from higher resource availability within their parent's relatively high quality habitat compared to what they would encounter if they dispersed (Duffield and Bull, 2002). However, our results suggest no 'resource access' benefits, as parent-offspring associations tended to form on low quality habitat sites. Characterization of habitats occupied by offspring associated versus not associated with their parents, and detailed observational studies that look at how parental presence influences offspring foraging behavior are potential research directions to consolidate our understanding in this system of the mechanisms underlying the positive effect of mother-offspring association on offspring growth and body condition. Alternatively, the increased offspring growth and body condition may be a result of parental effects as opposed to benefits acquired as a result of association *per se*; however, we did not find that parents in better body condition (as a proxy of parental quality) were more likely to associate with their offspring.

Despite benefits of maternal association for offspring growth, we did not find any benefits for survival. This was surprising given that one of the key hypotheses for the benefits of parent-offspring association in the *Egernia* lineage of lizards is protection from conspecific infanticide (Langkilde et al., 2007; Sinn et al., 2008). Our results instead suggest that parental tolerance of offspring has the primary function of enabling a safer and more efficient foraging environment, as opposed to direct protection from conspecifics. In support of this conclusion, *L. whitii* and other species of *Egernia* have been shown to tolerate their own, but not unrelated offspring, within their home range

(O'Connor and Shine, 2004; While et al., 2009a); if parental-offspring association has low costs, the presence of unrelated offspring may nevertheless negatively affect a parent's own offspring through competition over resources within the parent's habitat. The observation of parental aggression towards unrelated offspring fits this 'resources or foraging benefits' hypothesis. If parental-offspring association has benefits for protection from infanticide, we would expect no parental aggression towards unrelated offspring, as this should not increase the risk of infanticide to the parent's genetic offspring (and may even reduce it, through a dilution effect).

We found no costs to parents of associating with offspring. This was true when costs were measured both in terms of body condition or marks of aggression suffered. This is consistent with studies on reptiles more broadly where the costs associated with the early stages of parental care are often small (Aubret et al., 2005; Huang, 2007; Stahlschmidt et al., 2012). An absence of costs associated with increased parent offspring association may help facilitate the evolution of more complex forms of care because it promotes a kin structure that could favor the expression of more costly behaviours (for example, parental provisioning). However, similar to many other studies, the lack of costs to parents may be because true fitness costs are difficult to detect from a single season analysis (reviewed in Alonso-Alvarez and Velando, 2012) and with low sample size (Graves, 1991). Thus, more data on the long-term consequences of increased parent-offspring association for both parents and offspring is required.

This study has provided evidence for effects of habitat structure on the extent of parental-offspring association within *L. whitii*. We believe that such a simple increase in parental-offspring association may be characteristic of the early stages of the evolution of complex forms of parental care and group living. When the costs to care are low, parents will tolerate offspring, facilitating prolonged associations between parents and offspring. This enhanced kin association sets the foundation from which more complex care behaviors can emerge. The *Egernia* lineage show variation between populations and species in the environments they inhabit and the degree to which they associate with offspring (from no care in species such as *L. inornata*, to extended family groups with multiple cohorts of offspring cared for in *E. cunninghami* – reviewed in Chapple, 2003; While et al. 2015). These species therefore offer opportunities to connect within species patterns between ecology and parent-offspring association with the emergence

and diversification of more complex forms of parental care across species in the *Egernia* lineage. Ultimately this will provide a greater understanding of the casual effects of specific ecological conditions on the emergence of parental care more broadly.

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Chapter Four

Habitat Saturation Promotes Delayed Dispersal in a Social Reptile

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Abstract

When and where offspring disperse has important implications for the evolutionary emergence and maintenance of group living. In non-cooperative breeders, direct benefits of delayed dispersal are relatively limited, suggesting that decisions regarding whether or not to remain in the parental territory are largely driven by the availability of suitable habitat in which to settle. While there is ample evidence of correlations between habitat saturation and delayed dispersal, experimental tests are rare, particularly for species with facultative group formation. We manipulated the density of conspecifics in enclosed populations of a family living reptile to experimentally evaluate the influence of habitat saturation on the tendency to delay dispersal. Habitat saturation did not influence whether or not offspring explored their surroundings. However, when conspecific density was high, more offspring delayed dispersal and those that did settle in high-density enclosures had reduced survival. These patterns appear to be due to increased dispersal costs imposed by conspecific aggression; offspring that explored high-density enclosures had reduced body condition and a greater risk of mortality. We discuss these results in the context of the evolutionary origins of family living.

Introduction

When and where to disperse are decisions faced by offspring that have significant implications for the size and stability of family groups and ultimately the opportunity for selection on cooperative behavior between kin (Spinks et al. 2000; Le Galliard et al. 2003; Lucia et al. 2008). Indeed, delayed dispersal and cooperation represent a fundamental co-evolutionary feedback in the emergence and elaboration of complex social systems (Le Galliard et al. 2005). A crucial step in understanding the origin and maintenance of group living is therefore to understand factors that mediate offspring dispersal.

Dispersal decisions are shaped by the costs and benefits of dispersal and philopatry. Dispersing is often crucial to avoid competition with kin (Hamilton and May 1977), but it also carries costs. In particular, if settlement is limited by extrinsic factors, such as a shortage of suitable habitat (Komdeur 1992; Walters et al. 1992), a shortage of mates (Pruett-Jones and Lewis 1990), a low probability of successful independent breeding (Emlen 1982; Stacey and Ligon 1987), or enhanced mortality of dispersers (Heg et al. 2004; Bach et al. 2006). Dispersal entails additional costs when offspring forfeit benefits associated with philopatry, including nepotistic access to resources (Ekman and Griesser 2002; Dickinson and McGowan 2005), protection from conspecific aggression (Black and Owen 1987; O'Connor and Shine 2004) or predation (Griesser and Ekman 2005; Griesser et al. 2006), and the inheritance of parental territories (Ekman et al. 2001; Kokko and Ekman 2002). Recent research suggests that benefits, constraints and particular life history characteristics act in concert to bias the cost-benefit trade-off of dispersal and create demographic conditions conducive to philopatry (Covas and Griesser 2007; Hatchwell 2009). Indeed, the selection dynamics surrounding dispersal are often complex, involving multiple selection pressures (Perrin and Lehmann 2001; Kokko and Ekman 2002; Matthysen 2012), many of which can operate in context- or phenotype-dependent ways (Bowler and Benton 2005; Clobert et al. 2009).

The relative contribution of the costs and benefits of dispersal also vary between different systems. For example, the benefits of philopatry are particularly relevant to cooperative breeders, which may gain more in terms of inclusive fitness from becoming helpers-at-the-nest than from becoming opportunistic floaters or establishing a

breeding territory in sub-optimal habitat (Koenig et al. 1992; Komdeur 1992; Cockburn 2013). However, for non-cooperatively breeding species, benefits of philopatry are often restricted to direct benefits associated with family living (see above), which are less likely to outweigh the costs of kin competition (Hamilton and May 1977; Perrin and Lehmann 2001; but see Kokko and Ekman 2002). This lack of indirect fitness benefits means that delayed dispersal can easily yield a net loss to inclusive fitness in non-cooperative species (Ronce et al. 1998; Ridley and Sutherland 2002; Gardner et al. 2003). For delayed dispersal to occur under these conditions the costs of dispersal must therefore be heavily compounded by extrinsic factors, suggesting a major role of ecological constraints.

Lizards do not breed cooperatively, but can nevertheless form large complex social groups (Doody et al. 2012; Gardner et al. 2015). For example, species of the Egerniinae (hereafter *Egernia)* represent a continuum of social complexity ranging from solitary to nuclear families to communal family groups, characterized by varying degrees of delayed dispersal (Chapple 2003; Gardner et al. 2015). Targeted experiments within this system offer the opportunity to ask fundamental questions about 1) what drives variation in delayed dispersal and 2) how this may have played a role in the diversification of social systems across the lineage. Here we examined the influence of ecological constraints on offspring dispersal behaviour in a facultatively social lizard, *Liopholis whitii*. *Liopholis whitii* (previously *Egernia whitii*) is a viviparous lizard that forms socially monogamous pair bonds and displays intra-familial variation in offspring dispersal behavior. Specifically, one or more offspring from a litter can delay dispersal for an extended period of time, residing within the parental home range for up to a year (While et al. 2009a). We used a large-scale captive population experiment to test the influence of habitat saturation on offspring dispersal behavior in *L. whitii* by manipulating the density of conspecifics in available habitat (henceforth habitat saturation). This approach allowed us to directly test the causal relationship between habitat availability and delayed dispersal, providing insight into the factors which may have promoted the initial origins of family living in this taxa.

Materials and Methods

Study species

Liopholis whitii is a medium sized (<100mm snout-vent length [SVL]) viviparous skink found in dry habitats throughout southeastern Australia. The majority of adults live in small family groups centered on a stable, socially monogamous pair-bond and one or more offspring (Chapple and Keogh 2005; Chapple and Keogh 2006; While et al. 2009a). Mating takes place during the austral spring (September–October) and gestation lasts 3–4 months, with births occurring in the Austral summer (January-February) (While et al. 2007).

Animal capture and husbandry

During November 2014 we captured gravid and non-gravid females from a population at Orford on the east coast of Tasmania, Australia (42°57'S, 147°88'E) using both 'mealworm fishing' and noosing techniques and transported them back to university facilities to be housed until birth. In the laboratory we measured individuals for snout-to-vent length (SVL), total length, head length, head width (±1 mm) and mass (±1 mg). Animals were toe-clipped for unique identification.

Adults were housed individually in temperature- and light-controlled rooms, with room lights set to a natural (12:12) day-night cycle. Terraria (30x60x40cm) were made from opaque plastic and contained ~5cm of paper-based cat litter, food and water dishes, a shelter at one end and a basking rock at the other. Basking lamps were set to come on 1 hour after ambient lights came on (simulating sunrise) and turn off 1 hour before they went out (sunset). This provided a thermal gradient of 17-40°C in the terraria, allowing animals attain their preferred body temperature of 34°C (Bennett and John-Alder 1986). Water was provided *ad libitum* and animals were fed every two days on mealworms dusted with mineral supplement and periodically with pureed fruit. Animals were held under these conditions until they gave birth. From January 2015, the terraria of gravid females were checked daily for offspring. Upon discovering offspring, date of birth was recorded and the individual was temporarily separated from its mother to obtain weight (± 10 mg), SVL (± 0.5 mm) and total length (± 0.5 mm). Each offspring was then toe-clipped for unique identification and its birth order in the litter recorded (i.e., 1st born, 2nd born etc.). Females that gave birth to a single offspring were

excluded from the experiment in order to maximize sample size and allow us to investigate the effects of birth order on dispersal behavior. The 30 females used as mothers in the experiment gave birth to a total of 88 offspring. Clutch size ranged from 2 to 4 (average 2.9 ± 0.1, $n_2 = 8$, $n_3 = 16$, $n_4 = 6$). Average within-clutch birth spread was 3.8 ± 0.3 days, ranging from 1 to 8 days between females.

Enclosure Experiments

Following birth, we relocated females and their newly born offspring to large semi-natural outdoor enclosures at the Cambridge farm facility and housed them there over a period of two months (February-April). Each experimental replicate was comprised of two adjacent 8 x 8 meter enclosures with identical resources and separated by a partition, creating 6 pairs of one 'home' and one 'dispersal' enclosure. We installed three dispersal gates made of 8mm mesh in the partition separating each enclosure pair, allowing offspring to move between enclosures within a pair but prohibiting the movement of adults. Each enclosure was provided with identical resources. Five wooden pallets, each covered with ~100L of burrowing substrate and topped with cement bricks facing each major aspect, were spaced evenly throughout each enclosure, creating 5 high quality crevice sites. This design allowed us to manipulate the degree of habitat saturation in dispersal enclosures by introducing a variable number of adult conspecifics.

We released five females and their offspring into each 'home' enclosure, giving a total of 30 mothers and 88 offspring included in the experiment. This meant that home enclosures were entirely saturated, with one habitat patch for each female and her offspring. Before release, we attached numbered cloth stickers (Tesa, Germany) to the back of each adult female and a colored bee tag fixed with non-toxic glue (Pender Beekeeping Supplies) to each offspring for identification. The bee tags were colour coded with five different colours corresponding to each of the five mothers within a home enclosure. We used the position of a tag along an offspring's back to differentiate which member of a litter it was (i.e., tag fixed to neck = 1st born offspring, middle of the back = 2nd born, pelvis = 3rd born, base of tail = 4th born) and therefore its unique ID.

We imposed treatments by manipulating the density of adult conspecifics in each 'dispersal' enclosure, producing two treatments of three replicates: 1) a low-saturation treatment where two females were released into the 'dispersal' enclosures and 2) a high

saturation treatment where four females were released into the 'dispersal' enclosures. Thus, we use the term habitat saturation to refer to population size relative to available resources (sensu Stacey and Ligon 1987), or relative density (e.g. Koenig et al. 1992). All animals allocated to a given enclosure block were released simultaneously. However enclosure releases themselves were staggered over three weeks due to the temporal spread of births between females. As all individuals were re-captured at the same time in April, the duration of the experiment differed between enclosure blocks, ranging from 7 to 10 weeks. Therefore, we alternated releases between treatments to avoid any confounds between treatment and experimental duration. Mothers and their offspring were randomly allocated to treatments before release, resulting in 43 and 45 offspring in the low and high saturation treatments respectively. We did not observe any adult deaths throughout the experiment or find any adult corpses during recapture, however three adults from separate enclosures (all from home enclosures; 2 from low and 1 from high saturation treatment) could not be recovered at the end of the experiment.

Behavioural observations

We collected positional data and behavioural interactions on all visible individuals twice a day in a morning and afternoon observation session. In each of these sessions we spent 20 minutes observing each enclosure block, recording which enclosure each individual was in ('home' or 'dispersal') and their physical position within the enclosure. For behavioural observations we recorded two classes of interactions: positive interactions in which individuals were observed basking in physical contact or within a body length of each other with no signs of aggression; and negative interactions in which individuals were observed to chase, bite or wrestle one another (sensu Halliwell et al. 2016). Afternoon observation sessions commenced at least 4 hours after the completion of morning sessions to reduce spatial auto-correlation. At the end of the experiment we recaptured all mothers and offspring and returned them to the laboratory to measure size, mass and body condition before releasing them back to their initial capture location.

Estimating home ranges and maternal overlap

During two months we recorded 1365 positional observations. The average number of observations for adults and offspring during our experiment was 20.6 ± 1.3 and 8.5 ± 0.9 respectively. For adults, we used observational point data to calculate 50% kernel contours via least-squares-cross-validation (LSCV) in the computer program Ranges 9 (Anatrack 2016). We set a constant LSCV smoothing factor of 0.75 when generating kernels as this was most effective at buffering against over-smoothing and under-smoothing of kernels at the extremes of the sample size range (see Kie 2013). Due to a relatively low number of observations for offspring we were unable to confidently estimate home range kernels, preventing us from assigning delayed dispersal based on mother-offspring kernel overlap. Instead, we used the 50% kernel of each female to identify which crevice site she had utilized as her primary retreat site. After identifying sites occupied by each female, we defined juveniles as overlapping with their mother if 50% or more of their observations were on or within one meter of that crevice site. This corresponds with the distribution of offspring locations around female crevice sites in the field (While et al. 2009b; GM While unpublished data). For females with 50% kernels including more than one crevice site, observations of juveniles falling within one meter of any of these sites were included. Assignment of overlap among those offspring for whom we had enough data to calculate kernel home range (offspring who reached 80% of the home range asymptote; Rose 1982, n = 11), was the same whether using the above method or that based on overlap of home range kernels. Offspring assigned as overlapping with their mother based on our point data method had an average of 79.8 ± 0.04% of their observations falling within this range, compared to 12.2 ± 0.02% for offspring assigned as not overlapping their mother. We excluded offspring with fewer than 3 observations (n=24) from all analyses except those taking 'explored' and 'survived' as response variables, as the reliability of these responses did not depend on total observation number; a single observation of an individual in the dispersal enclosure is proof of exploration and the recapture of an individual at the end of the experiment is proof of survival. Furthermore, 23 of the 24 offspring excluded from other analyses died early in the experiment, explaining the lack of observations but also providing important data for the survival analysis. Thus, the average number of

observations for offspring included in analyses taking 'explored' and 'survived' as the response was 8.5 ± 0.85, and for all other analyses was 11.5 ± 1.0.

Determining exploration, dispersal and parent-offspring associations

We defined two non-exclusive classes of dispersing individuals, coded as binomial variables, for the purpose of statistical analyses. *Explorers* were offspring who were observed within the dispersal enclosure at least once during the experiment. *Settlers* were offspring observed in the dispersal enclosure in at least three sequential observations and never observed back in the home enclosure. Preliminary data analysis showed that three sequential observations was sufficient to identify settlers. Specifically, out of 16 individuals who were observed in the dispersal enclosure on three sequential occasions, and for whom we have additional observations following this period, only one was observed to return to the home enclosure, representing a 93.5% chance of our criterion correctly identifying a settler.

Delayed dispersal implies a persistent association between parents and offspring within the natal range. Therefore, offspring were only considered to have delayed dispersal if they overlapped their mother's core home range area (see above). We further reduced this data set to include only offspring who both overlapped and were also observed interacting positively with their mother (number of observed interactions ranged from 1-7). These positive associations were easy to identify as offspring were often seen basking directly on top of their mother. Combined, these criteria revealed rates of delayed dispersal comparable to that observed in natural population studies (While et al. 2009a) and experimental contexts (Botterill-James 2016), with 23.9% of offspring (21 offspring from 13 of the 30 clutches) classed as delaying dispersal and 10.2% (9 offspring from 9 of 30 clutches) classed as displaying positive associations. Importantly, while offspring classified as explorers could either go on to settle or delay dispersal, these were not the only possible behavioral responses; offspring could establish a home range within the home enclosure that did not overlap that of their mother, resulting in a negative response for both these categories. Furthermore, settle and delay dispersal were treated as mutually exclusive behaviours; any offspring classified as having settled could not be considered to have also delayed dispersal. This classification was never contentious, as offspring who settled never had ≥50% of their observations within the maternal home range.

Statistical analyses

We conducted all statistical analyses in R (Version 3.3.0, 2016), using linear mixed models (LMMs) and generalized linear mixed models (GLMMs) fitted by standard methods in the 'lme4' package (Bates et al. 2014). For LMMs, the significance of fixed effects are reported based on *F*-tests with degrees of freedom approximated by the Kenward-Rogers method. For GLMMs, the significance of fixed effects are reported based on Wald chi-squared tests. To investigate the influence of treatment and phenotypic traits (SVL and release body condition) on offspring dispersal behavior we fit four separate binomial GLMMs taking whether or not offspring explored, settled, delayed dispersal and formed positive associations as response variables. We began by including all main effects as well as all pairwise interactions between treatment, SVL, release body condition and birth order as predictor variables. If interaction terms were non-significant ($P > 0.05$) they were dropped from the model and results from reduced models are presented. We defined the body condition of each individual from the residuals of a least squares linear regression of body mass on SVL. Body condition values were generated at two different time points, once before release into the enclosures and again upon recapture at the end of the experiment.

To examine whether the costs associated with exploration were context-dependent we tested for treatment by exploration interaction effects on growth (i.e. change in SVL) and recapture body condition using Gaussian GLMMs, and survival using a binomial GLMM. For these models we included the pairwise interaction and main effects of treatment and explore as well as the main effect of delayed dispersal as predictors. Release body condition and release SVL were included as covariates were necessary (Tables 4.1 and A3.1). We re-ran all models with positive association instead of delayed dispersal as a predictor to test whether positive interactions with the mother, above and beyond simple home range overlap, were necessary for benefits of philopatry. Due to the low occurrence and survivorship of settlers in the high saturation treatment (see results) we were unable to formally test for treatment by settlement interaction effects on growth, survival or body condition.

Throughout our analyses we included offspring age and experimental duration (number of days spent in the enclosure) as covariates where relevant to control for the temporal spread of birthing between females and staggered release of animals into enclosures, respectively (See Tables 4.1 and A3.1 for full model specifications). We also

included enclosure ID as a random factor in all analyses to account for block effects. We checked all data for violations of model assumptions, including homogeneity of slopes where covariates were used, and all model fits for over-dispersion using a Pearson's chi-squared test. Means and standard errors are reported throughout.

Results

Ecological constraints on dispersal behavior

Habitat saturation had no effect on the probability that offspring explored dispersal enclosures (Table 4.1; Figure 4.1). However offspring were less likely to settle in dispersal enclosures in the high compared to the low saturation treatment (Table 4.1; Figure 4.1). Offspring that settled in dispersal enclosures were also of smaller body size than those that remained in the home enclosure (Table 4.1).

Delayed dispersal, whereby offspring established within their mother's core home range area, occurred at twice the rate in the high (14 offspring) versus low saturation (7 offspring) treatment (Table 4.1). Delayed dispersal was also positively associated with offspring SVL (Table 4.1). A significant treatment by body condition interaction indicated that offspring that delayed dispersal in the high saturation treatment were those in poorer body condition at the start of the experiment, whereas this was not the case in the low saturation treatment (Table 4.1; Figure 4.2). A similar effect was found for offspring who exhibited positive associations with their mother (Table 4.1). Birth order had no effect on any aspect of dispersal behaviour (Table 4.1).

Costs of dispersal and benefits of philopatry

Out of 88 offspring, 42 survived until the end of the experiment, producing an offspring pre-winter survival rate (48%) comparable to that seen in natural populations (While et al. 2009) and other captive population experiments (Botterill-James et al. 2016). Exploration was associated with an increase in mortality as well as a loss of body condition among offspring in the high saturation but not the low saturation treatment (treatment*exploration interaction, mortality: $\chi^2 = 4.67$, $P = 0.03$; body condition: $F_{1, 31.2} = 8.97$, $P < 0.01$; Figure 4.3), indicating elevated costs of exploration at high conspecific density. Habitat saturation also influenced the survival of those individuals that

eventually settled dispersal enclosures. Specifically, just one settler survived until the end of the experiment (25% success rate) in the high saturation compared to 8 in the low saturation treatment (73% success rate).

We found no benefits of delayed dispersal itself in terms of offspring growth, body condition or survival (Table A3.2). However, offspring that displayed positive associations with their mother were in better body condition upon recapture ($F_{1, 32.7}$ = 5.72, P = 0.02). In contrast, positive associations did not affect the growth (ΔSVL; $F_{1, 33.4}$ = 0.15, P = 0.7) or survivorship (χ^2 = 0.39, P = 0.53) of offspring.

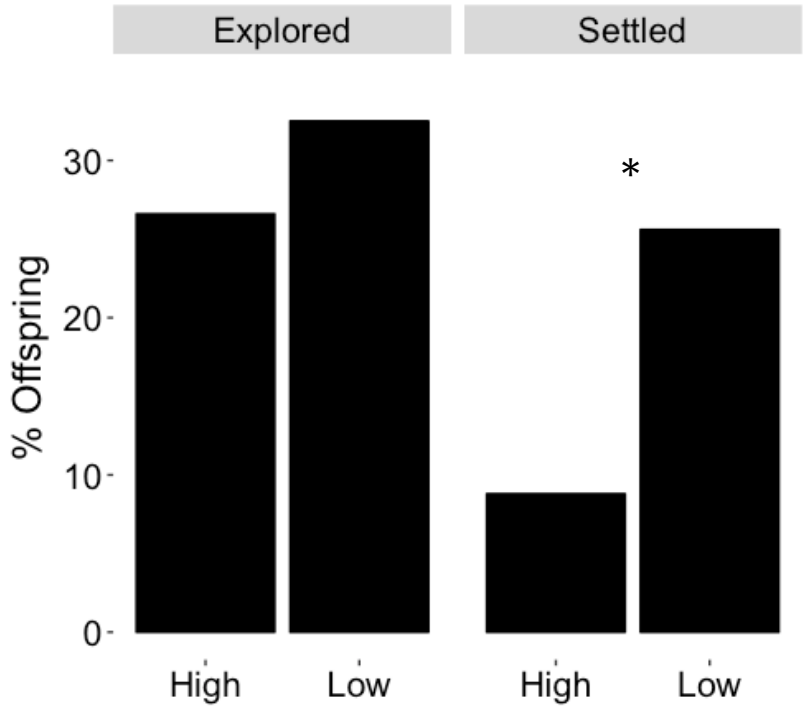

Figure 4.1. Proportion of total offspring from the high and low saturation treatments that explored and settled in dispersal enclosures. Asterisk indicates a significant difference between treatments in the probability of settlement. See Table 4.1 for test statistics.

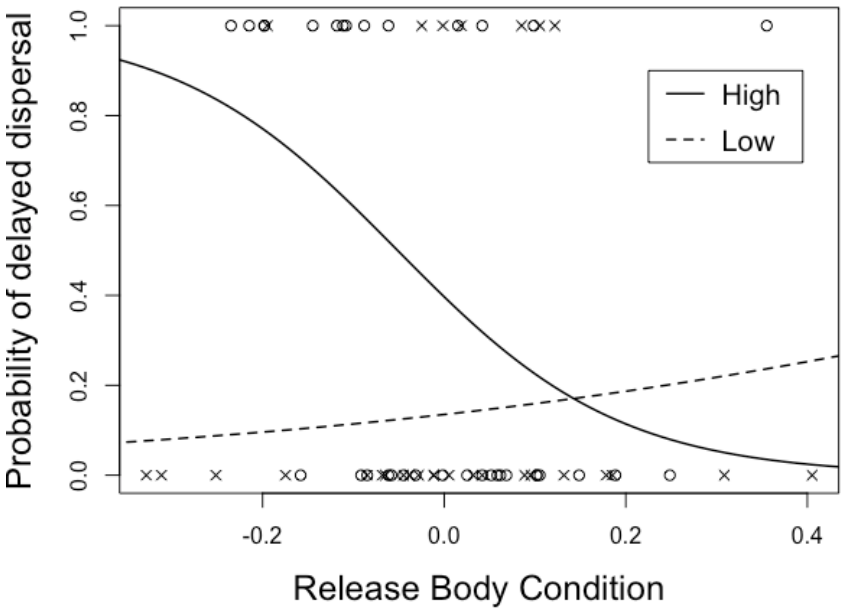

Figure 4.2. Fitted values of the probability of delayed dispersal in relation to release body condition in the high saturation (circles, unbroken line) and low saturation (crosses, broken line) treatments. Probability estimates derived from the binomial GLM described in Table 4.1. Fitted lines indicate a significant treatment by release body condition interaction effect in which offspring in the high saturation treatment were more likely to delay dispersal when they had poor body condition upon release. Analyses restricted to individuals with >3 observations (see methods).

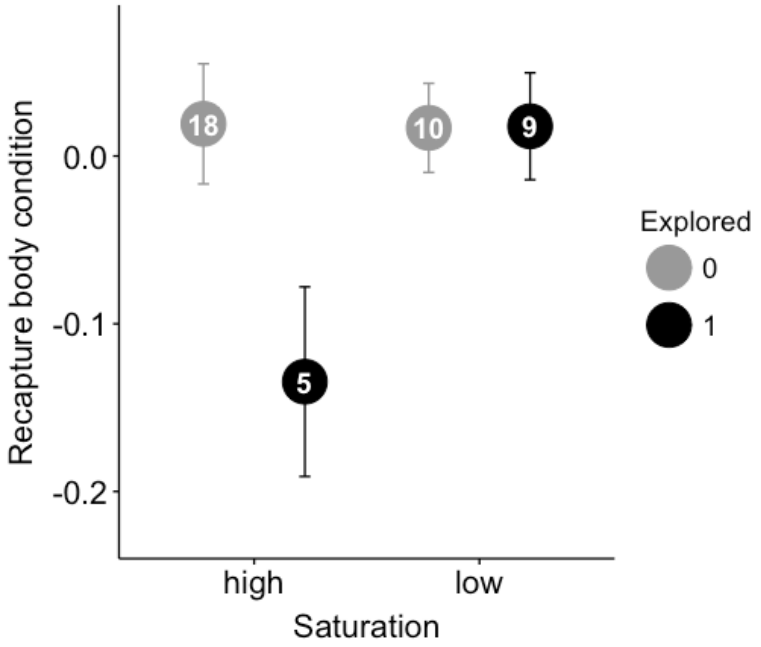

Figure 4.3. Body condition upon recapture of offspring from the high and low saturation treatments that were and were not observed to explore the dispersal enclosure. Data restricted to individuals that survived until recapture at the end of the experiment. Numbers within points indicate sample size for each group.

Table 4.1. The effect of treatment, body size (SVL) and initial body condition on different aspects of offspring dispersal behaviour. Significant terms shown in bold. Marginally non-significant terms indicated by a '•' symbol. Dashes indicate interaction terms that were dropped from the model after being found non-significant.

Variable	Explored	Settled	Delayed dispersal	Associated Positively
Treatment	$\chi2 = 0.497$, P = 0.481	**$\chi2 = 8.304$, P = 0.004**	**$\chi2 = 4.595$, P = 0.032**	$\chi2 = 0.240$, P = 0.624
SVL	$\chi2 = 0.006$, P = 0.938	**$\chi2 = 4.297$, P = 0.038**	**$\chi2 = 5.270$, P = 0.022**	$\chi2 = 0.225$, P = 0.635
Release body Condition	$\chi2 = 0.279$, P = 0.597	$\chi2 = 0$, P = 1	**$\chi2 = 4.479$, P = 0.034**	$\chi2 = 3.251$, P = 0.071•
Birth Order	$\chi2 = 0.501$, P = 0.919	$\chi2 = 4.464$, P = 0.215	$\chi2 = 3.004$, P = 0.391	$\chi2 = 0.279$, P = 0.964
Treatment * Release body Condition	-	-	**$\chi2 = 3.871$, P = 0.049**	**$\chi2 = 4.435$, P = 0.035**
Age (covariate)	**$\chi2 = 4.721$, P = 0.030**	**$\chi2 = 4.686$, P = 0.030**	$\chi2 = 2.541$, P = 0.111	$\chi2 = 1.323$, P = 0.250

Discussion

Our results provide clear experimental evidence that dispersal behaviour and the success of dispersal attempts are directly affected by habitat saturation in a family living lizard. Offspring showed a willingness to disperse when conspecific density was low and habitat vacancies were available, but delayed dispersal when conspecific density was high and when unoccupied habitat was limited. Dispersal movements were also more costly under these conditions, highlighting the role of conspecific aggression in mediating offspring dispersal decisions. Taken together these results establish an important link between ecological conditions, delayed dispersal and social organisation.

Habitat saturation did not influence whether or not offspring explored the dispersal enclosures, but it did influence the probability that offspring settled in those enclosures. This suggests a period of cost evaluation during exploration and a willingness to disperse and settle when nearby conditions were favourable. Reductions in relative body condition and an elevated risk of mortality associated with exploration in the high saturation treatment also indicate that the costs of exploring novel habitat increase with increasing conspecific density (e.g. Kingma et al. 2016). These mortality effects are likely be mediated by conspecific infanticide as adult *L. whitii* vigorously defend their territory from conspecifics (Sinn et al. 2008) and actively attack unrelated juveniles (pers obs. G While; B Halliwell), increasing the risks of injury or mortality associated with dispersal movements. Indeed, conspecific aggression is a major cause of offspring mortality in other group living *Egernia* (Lanham and Bull 2000; O'Connor and Shine 2004) and likely contributes to a suite of traits that predispose species of this group to delay dispersal. Costs incurred by intra-specific competition are also often attributable to competition for food or space use. For example, juvenile *Lacerta vivipara* that settle in habitat patches free from conspecifics grow faster and reproduce earlier than those who settle in patches occupied by conspecifics (Le Galliard et al. 2005; also see Lecomte et al. 1994). Although similar processes are likely to operate in *L. whitii*, we were unable to test for treatment effects on the recapture body condition of settlers due to markedly reduced survival among offspring that settled in high saturation enclosures.

The reduced propensity for offspring to settle in dispersal enclosures in the high saturation treatment corresponded with an increased incidence of delayed dispersal and mother offspring association. This suggests a crucial link between habitat

saturation, dispersal costs and delayed dispersal; not only does habitat saturation increase the costs of dispersal, it also increases the incidence of philopatry. Taken together these results provide strong evidence for a causal link between ecological constraints and social organisation. Lucia and colleagues (2008) demonstrated a similar relationship between conspecific density and delayed dispersal, group formation and group size in prairie voles, *microtus ochrogaster*, arguing that ecological conditions which influence the social structure of populations may facilitate evolutionary transitions in social complexity. We have previously suggested that these processes could have contributed to divergence in social organisation across the *Egernia* skinks (While et al. 2009b; While et al. 2014). Unfortunately, the short duration of this experiment precludes us from examining long-term consequences of dispersal behaviour on offspring fitness directly. Importantly, however, delayed dispersal may have evolutionary consequences whether or not it presents immediate benefits to offspring; increased association between parents and offspring has the potential to uncover plastic responses in behaviour and allow selection to act on beneficial interactions when they do occur, setting the stage for the emergence of more complex forms of parental behaviour (Uller and Helanterä 2014; While et al. 2014).

What then are the phenotypic predictors of delayed dispersal? Our results indicate that birth order has a negligible effect on offspring dispersal behaviour in this species. This is in line with previous research showing an overall limited effect of birth order on offspring phenotype and survival (While and Wapstra 2008) suggesting that birthing asynchrony may be a mechanism that alters the completive environment within the litter as opposed to the competitive ability of offspring *per se* (While and Wapstra 2008). In contrast, we found body size had a significant effect on offspring dispersal, with small offspring more likely to settle in dispersal enclosures and large offspring more likely to delay dispersal. Furthermore, offspring in poor body condition were more likely to both delay dispersal and display positive associations with their mother, but this effect was only observed in the high saturation treatment. Other studies on lizards have shown that kin competition can promote dispersal of offspring in good body condition, because these individuals are better able to bear the costs of dispersal (Léna et al. 1998; Cote et al. 2007; Cote and Clobert 2010). Therefore one possible explanation for this treatment by body condition interaction is that the costs of dispersal were so heavily reduced in the low saturation treatment that settlement

became a feasible strategy for offspring in poor condition, eroding the relationship between body condition and delayed dispersal observed in the high saturation treatment. Additional data on the phenotypic correlates of dispersal from natural populations of *Liopholis whitii*, as well as other *Egernia* species, will shed further light on this topic.

Benefits of philopatry are well documented among the *Egernia* group. For several species, delaying dispersal to reside within the parental territory provides offspring with protection against conspecific harassment and infanticide (O'Connor and Shine 2004; Sinn et al. 2008; While et al. 2009b), an important source of offspring mortality (Lanham and Bull 2000; O'Connor and Shine 2004). This 'safe haven' effect also allows offspring to utilize key resources within the parental territory that they would otherwise be unable to defend (O'Connor and Shine 2004) and may also have physiological benefits, including re-direction of energy from dispersal, vigilance and burrowing behaviors to growth and development (Botterill-James et al. 2016). We found that offspring that delayed dispersal were in better body condition at the end of the experiment than those that did not delay dispersal. However, these benefits were restricted to individuals that also displayed positive social associations with their mother. This indicates the potential for variable fitness returns between offspring that delay dispersal that depend on the nature of their association with the mother. The mechanisms underpinning the observed differences are currently unknown. One possible explanation is that female *L. whitii* vary the degree of tolerance shown toward different offspring, resulting in differences in the extent to which individual offspring are able to access key resources within the maternal home range. Any benefits of philopatry would then depend on this process.

Offspring dispersal decisions are a product of social context and will be mediated by the resolution of conflict between different agents in the social group. For example, parent-offspring conflict can promote selection for maternal control of offspring dispersal (Starrfelt and Kokko 2010). Female *L. whitii* occasionally chase and even bite their own offspring (pers obs. G While; B Halliwell), suggesting that parental harassment could act as a mechanism to promote offspring dispersal (e.g. Masters and Shine 2003). However, previous captive population studies on *L. whitii* have failed to detect any costs to parents of tolerating offspring within their home range (Botterill-James et al. 2016), calling the motivation for parental harassment into question.

Alternatively, competition between siblings over access to the parental home range may underpin dispersal decisions in this system. Our finding that SVL was positively associated with delayed dispersal and negatively associated with settlement suggests an influence of competitive interactions in which larger offspring promote the dispersal of competitively inferior siblings. Finally, offspring may be born with or develop early in life, a behavioural phenotype that promotes or supresses dispersal behaviour (Cote and Clobert 2007; Vercken et al. 2007; Cote et al. 2010). Importantly, behavioral phenotypes can also be associated with specific morphological adaptations (O'Riain et al. 1996; Sinervo et al. 2006), making it unclear whether differences in the physical characteristics of offspring are a cause or a consequence of dispersal behavior. As our results do not allow us to disentangle the relative contribution of parents, siblings or offspring themselves as mediators of dispersal decisions, this provides an intriguing avenue for future research.

In summary, by manipulating conspecific density this experiment provides empirical evidence for a causal link between habitat saturation and delayed dispersal in a family living lizard. In turn these results support the hypothesis that variation in ecological conditions have been central to the diversification of social organization across the *Egernia* lineage. However, to truly understand the mechanistic drivers of dispersal behavior, we need to integrate knowledge of the costs and benefits of dispersal under different ecological conditions with that of life history traits generating demographic processes conducive to philopatry (Covas and Griesser 2007; Hatchwell 2009). Comparative studies of cooperative breeding birds suggest that long lifespan and high adult survivorship predispose species to delay dispersal by reducing the turnover of reproductive opportunities in the local environment (Arnold and Owens 1998; Arnold and Owens 1999). These life history traits are also characteristic of the *Egernia* (Chapple 2003) and may have facilitated a similar socio-ecological context conducive to the elaboration of social living. This combination of traits, combined with detailed life history data on a growing number of species, make the *Egernia* group uniquely suitable for comparative studies aiming to connect interspecific variation in social organisation to more general mechanistic drivers of divergence in social systems. Future work testing facultative responses in dispersal behavior to ecological constraints across species that vary in these key life history traits would be particularly valuable in addressing these aims. Thus, conclusions from this research may apply more broadly,

placing feedbacks between life history and ecology at the heart of social evolution in a range of vertebrate groups.

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Data Accessibility Statement

Analyses reported in this article can be reproduced using the data provided by Halliwell (2016).

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Section II

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Chapter Six

Correlated Evolution of Viviparity and Social Grouping in Squamate Reptiles

Manuscript status: Halliwell B, Uller T, Holland B, While GM (submitted) *Current Biology.*

Summary

Social groups are extraordinarily diverse in form and function across the animal kingdom. Whereas we understand well how many social systems are maintained, what predisposes certain lineages to evolve sociality in the first place is poorly resolved. Social associations between overlapping generations is a defining feature of many social groups, suggesting that delayed dispersal and parental tolerance of offspring is an important early step towards the emergence of more stable social organisation [1,2]. Giving birth to live young or attending eggs should increase opportunities for interaction between parents and related young, and may therefore promote a transition from solitary to group living [3]. Squamate reptiles (i.e., lizards, snakes and amphisbaenia) provide an opportunity to test this hypothesis since, unlike mammals and birds, live bearing and egg attendance have evolved many times in different lineages [4-6]. This diversity should generate considerable phylogenetic variation in the opportunity for reliable parent-offspring interactions and hence opportunities for natural selection to favour social tolerance of juveniles and ultimately group living. We found evidence for intergenerational social grouping of adults and juveniles in 98 species across 23 families of squamate reptile. These associations primarily involved offspring and adult females. Importantly, grouping was more likely to evolve in viviparous lineages than in oviparous lineages, including among oviparous species that attend their eggs. This is consistent with the hypothesis that stable social groupings in lizards and snakes emerge from tolerance of offspring rather than from aggregation of

unrelated individuals. Indeed, phylogenetic reconstructions revealed that 12 of the 14 transitions toward persistent social groups (consistent membership across multiple seasons or years) in lizards have occurred in viviparous lineages, suggesting live bearing as an important precursor in the evolutionary origins of group living.

Results and Discussion

Forms of Social Grouping

We found evidence for social grouping between adults and juveniles (henceforth social grouping) in 98 species across 23 families, including two species of amphisbaenia (Figure 6.1; Table A4.2). Groups varied in size, form, duration, as well as whether juveniles associated with females only or with adults of both sexes. Associations between group members ranged from passive tolerance of juveniles within adult home ranges to defense of offspring from conspecifics and predators [e.g. 7-9]. Social grouping was particularly common in Australian skinks of the *Egerniinae,* in which genetic studies have confirmed kin relationships among group members. Social associations in these species can last for several years and extend to mutual tolerance of multiple cohorts of offspring in large extended family groups [10]. Social grouping was less common and showed a more discrete phylogenetic distribution among snakes compared to lizards (Figure 6.1; Table A4.2). However, maternal attendance of offspring appears common in temperate pit vipers, particularly among the Crotalinae [8; Table A4.3].

Transitions to Social Grouping

We tested for the correlated evolution of social grouping and both viviparity and egg attendance using Bayesian and maximum likelihood methods (see supplemental experimental procedures) on a recently revised phylogeny of the squamates including 3951 species [11]. Social grouping occurred in viviparous species more than twice as often as in oviparous species (67 vs 30 species), despite the overwhelming majority of squamate species being oviparous [~80%

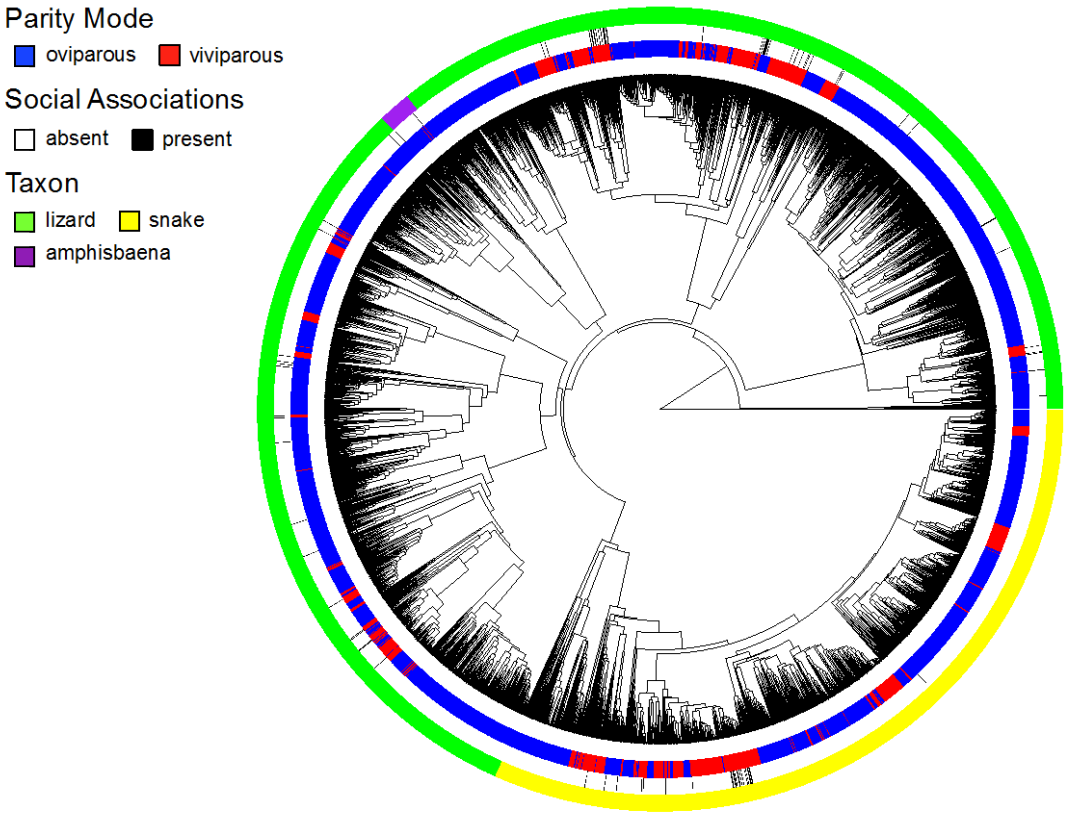

Figure 6.1. Phylogenetic distribution of social grouping across squamate reptiles. Phylogeny pruned [from 11] to contain all species for which parity data were available [n = 3951, *sensu* 5].

of species: 5]. Phylogenetic mixed modeling approaches revealed considerable phylogenetic structure in the distribution of social grouping (Table 6.1), indicating that social grouping has emerged in a number of key clades. After accounting for this phylogenetic structure, parity mode remained a highly significant predictor of grouping across species (Table 6.1). In contrast, there was no support for an effect of egg attendance on the occurrence of social grouping among oviparous species (Table 6.1). Alternative statistical approaches incorporating differential rates of speciation and extinction associated with each character state [12,13] provided strong support for these results (Table A4.1). Finally, ancestral state reconstructions clearly indicate correlated evolution of social grouping and viviparity (Figure 6.2), but not egg attendance (Figure A4.1). Indeed, while social groups containing adults and juveniles have arisen independently in both viviparous and oviparous lineages, transitions toward a state of social grouping occur at a considerably higher rate from a background of viviparity (Table A4.1), suggesting that viviparity has facilitated the evolutionary emergence of social associations between parents and offspring. This holds true

whether or not transitions in parity mode are bi-directional or constrained to be irreversible (i.e. oviparity to viviparity; Table A4.1).

These results suggest that the evolution of live birth may facilitate delayed dispersal in juveniles and increased social tolerance of juveniles in adults. Indeed, although social tolerance of juveniles may come with certain costs to adults, such as increased competition over resources and harassment by conspecifics, the costs of tolerating juveniles are generally low in squamates [14,15] and may easily be outweighed by benefits when juveniles residing on the territory are likely to be kin and mortality during dispersal is high or the availability of suitable habitat low. The localization of offspring within territories could then provide the foundation for the emergence of more complex forms of post-partum parental care [2,16]. For example, territorial behavior in non-caring ancestors appears to have facilitated the emergence of functional egg defense in *Eutropis longicaudata* [17]. However, our results suggest that in general, nest and egg attendance does not pre-dispose social associations between adults and juveniles to the same extent as live bearing. This may have an evolutionary explanation even if the advantages and disadvantages of social tolerance are similar. For example, viviparity ensures opportunities for physical association between parents and offspring at birth, whereas egg attendance only increases its probability. Furthermore, viviparity may facilitate kin recognition through processes operating in pre-partum development (e.g., via prolonged physiological exchange between mothers and offspring throughout gestation; [18-20]). Indeed, many viviparous squamates are capable of kin recognition [21-23], even after experimental separation from mothers and siblings at birth [24-26], whereas experimental demonstration of kin recognition is lacking in oviparous species [but see 27]

The association between adults and offspring promoted by viviparity should also facilitate the emergence of more complex social behavior. Indeed, phylogenetic reconstructions suggest that viviparity preceded the evolution of persistent social groups (consistent membership across multiple seasons or years) in 12 of the 14 independent origins of the trait (Figure A4.2), a result that conforms well to the observation that 17 of the 19 lizard species thought to exhibit stable, kin-based family groups are viviparous [28,29]. Thus, in promoting repeated social contact between generations, viviparity may set the stage for a gradual evolution towards more stable forms of social organisation and more advanced forms of post-partum care [16,30].

Although post-partum care in squamate reptiles rarely, if ever, extends beyond defense of offspring, our results support the hypothesis that increased parent-offspring interactions represent a particularly important initial step in the evolution of more complex and permanent forms of sociality [2,31-33].

Conclusions

Comparative analyses reveal that social groups involving adults and juveniles have evolved multiple times in squamate reptiles. Live bearing, but not egg attendance, appears to facilitate the emergence of social associations, suggesting that giving birth to live young promotes conditions conducive to selection for sustained parent-offspring interactions. These results imply that live bearing has provided an important pre-adaptive context for the emergence and stabilization of kin-based social organization in lizards and snakes and may provide the foundation from which more complex forms of social organization and behavior can evolve.

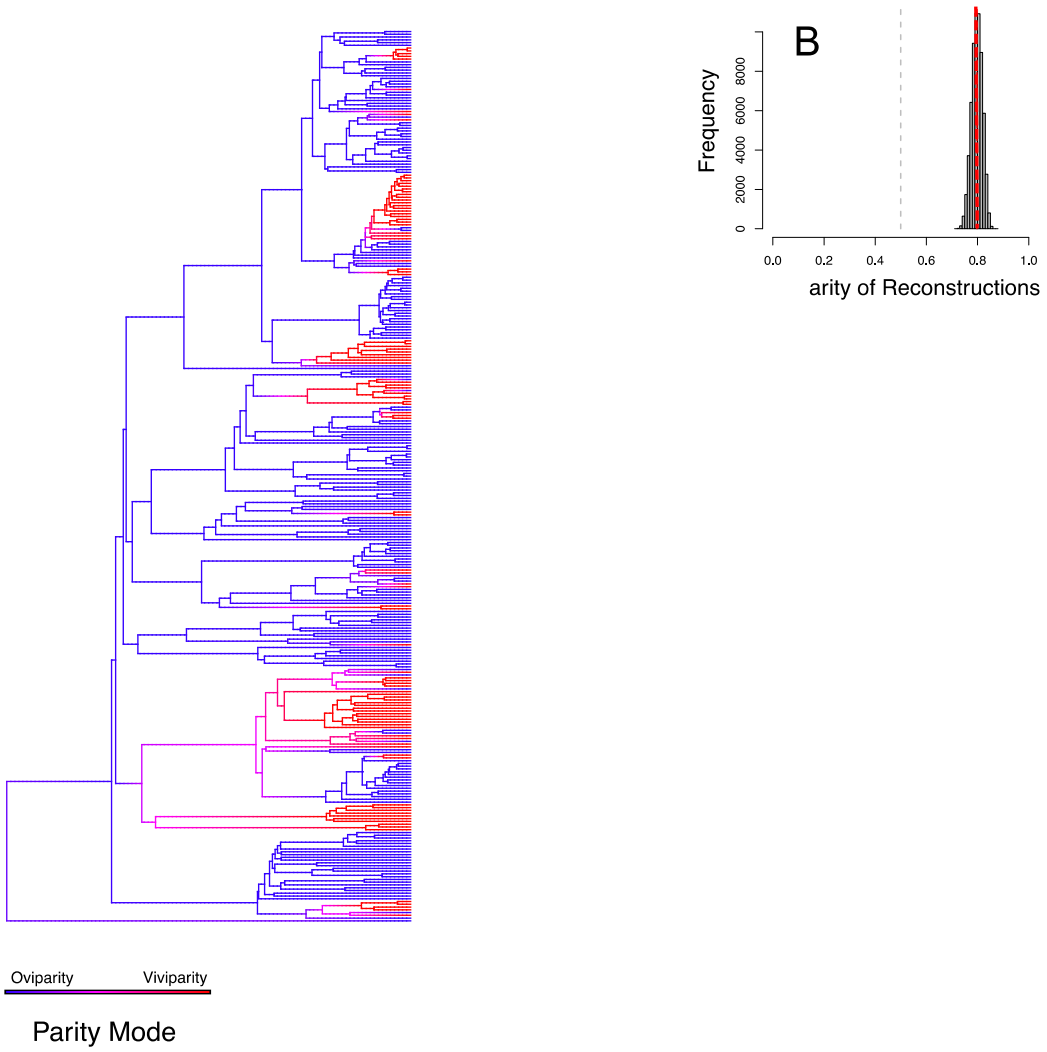

Figure 6.2. A: Correlated evolution of viviparity and social grouping among squamate reptiles based on ancestral state reconstruction by stochastic character mapping. Phylogeny restricted to species in the conservative data set (n = 324, see Supplemental Experimental Procedures). Branch colours represent posterior probability densities of edge states based on 1000 stochastic character maps of each reconstruction. **B:** Distribution of similarity scores between stochastic character map sets based on separate ancestral character state reconstructions of parity mode and social grouping. The grey line represents the null expectation of similarity between map sets if the two traits being compared show no evolutionarily correlation during reconstruction [36]. The red line represents the mean similarity between map sets based on our reconstructions.

Table 6.1. Phylogenetic generalized linear mixed models (PGLMM) testing the influence of parity mode and egg attendance on the occurrence of social grouping in squamate reptiles

	Data Set	Parameter	Estimate	Test Statistic	Confidence Interval 2.5%	Confidence Interval 97.5%	Bias
Parity Mode							
	Conservative (n = 324)	Intercept (β0)	-2.09 ± 1.02	**Z = -2.06, P = 0.04**	-3.8	-0.17	-0.11
		Parity Mode (β1)	2.46 ± 0.44	**Z = 5.62, P < 0.001**	1.72	3.18	0.14
		Citation Count	<0.001 ± 0.001	Z = 0.3, P = 0.764	-	-	-
		Signal in Residuals (s2)	3.18	**P < 0.001**	0.33	6.31	0.53
		Signal in Response (s2)	4.44	**P < 0.001**	-	-	-
	Relaxed (n = 1210)	Intercept (β0)	-3.33±1.54	**Z = -2.16, P = 0.031**	-5.06	-0.39	-0.95
		Parity Mode (β1)	2.98±0.52	**Z = 5.76, P < 0.001**	1.74	3.12	0.70
		Signal in Residuals (s2)	7.62	**P < 0.001**	1.88	4.48	4.56
		Signal in Response (s2)	9.13	**P < 0.001**	-	-	-
Egg Attendance (analyses restricted to oviparous species)							
	Conservative (n = 219)	Intercept (β0)	-1.61 ± 1.06	Z = 1.52, P = 0.13	-3.29	0.42	-1.61
		Egg Attendance (β1)	-0.947 ± 0.57	Z = -1.67, P = 0.095	-2.9	0.78	-0.94
		Citation Count	<0.001 ± 0.001	Z = -0.05, P = 0.96	-	-	-
		Signal in Residuals (s2)	3.05	**P < 0.001**	<0.001	3.42	3.01
		Signal in Response (s2)	2.67	**P < 0.001**	-	-	-
	Relaxed (n = 1049)	Intercept (β0)	-3.85 ± 1.55	**Z = 2.48, P = 0.013**	-4.95	-0.87	-1.1
		Egg Attendance (β1)	0.84 ± 0.66	Z = 1.28, P = 0.201	-0.58	2.46	0.11
		Signal in Residuals (s2)	7.48	**P < 0.001**	0.99	5.15	4.29
		Signal in Response (s2)	8.15	**P < 0.001**	-	-	-

N equals the number of species included in each analysis. The 'signal in response (s2)' parameter is derived from a model fit with no predictor variables and provides an estimate of phylogenetic structure of social grouping from each data set. Model estimates are reported ±SE. Confidence intervals of parameter estimates by parametric bootstrapping are derived from PGLMMs performed on simulated data (1000 and 250 simulations of the conservative and relaxed data sets, respectively; see Supplemental Experimental Procedures for details). Bias values represent the difference between parameter estimates from each real data model and the mean of the posterior of parameter estimates from simulations. Significant terms are shown in bold.

Experimental Procedures

Data Collection

We conducted extensive literature searches for all reports of parental care and social grouping in squamate reptiles, including both pre and post-partum care behaviours (see Supplemental Information for specific search terms). We also conducted individual searches on all species reported to show social groupings in an attempt to confirm reports yielded by initial searches. We treated social grouping as a discrete variable, coding it as 'present' based on reports of mixed-age aggregations, and 'absent' if substantial or targeted literature had failed to report presence of the trait (see Supplemental Information for more detailed description). We collected all data on the parity mode of species from supplementary materials in Pyron et al. [5]. We did not differentiate between viviparity and oviviparity, and excluded from analyses all species that were reported as displaying both oviparity and viviparity [e.g. *Zootoca vivipara*: 34].

Statistical Approaches

We used three different methods to test the hypothesis that social grouping has evolved more readily from a background of viviparity: binary phylogenetic generalized linear mixed modeling implemented in the R package 'ape' [35], Multi-state Speciation and Extinction models [13] implemented in the R package 'diversitree', and ancestral state reconstructions via stochastic character mapping implemented in the R package 'phytools' [36]. We also used stochastic character mapping to reconstruct the evolutionary history of transitions to stable social groups in the squamates. We used a recently published time-calibrated squamate phylogeny [11], including the tuatara, *Sphenodon punctatus,* as an out-group in all analyses. Due to challenges arising from incomplete data coverage, we performed analyses on two alterative data sets (conservative and relaxed) to confirm qualitative consistency of results (see Supplemental Experimental Procedures for full description and model outputs).

Author Contributions

GMW, B. Halliwell and TU conceived of the study. B. Halliwell conducted literature searches, data collation and statistical analyses. B. Holland provided consultation on statistical approaches and contributed to statistical analyses. B. Halliwell, GMW. and TU prepared the manuscript with assistance from B. Holland.

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Chapter Seven

General Discussion

This thesis took an integrated approach, linking experimental work and comparative analyses, to understand how feedbacks between behaviour and ecology influence the emergence and maintenance of social behaviour in a family living lizard. In this section I summarise the contribution that this body of work has made in advancing our understanding of social evolution. I begin by discussing my results in the context of classical theoretical predictions and go on to outline the ways in which this work may inform our understanding of the emergence and diversification of social behaviour in *Liopholis whitii*, the broader *Egernia* group, and across taxa more generally.

Ecological influences on social and mating behaviour in *Liopholis whitii*

The work of Emlen and Oring (1977) fundamentally shaped how we conceive of social and mating systems, revealing them to be emergent properties of interactions between a population's biology and its environment. However, despite representing a central theorem of mating system ecology, relatively few studies have experimentally tested the predictions generated by their model. My results showed that the aggregation of high quality habitat in combination with female territoriality fundamentally influenced the mating system (chapter 2). Furthermore, this had important implications for patterns of reproductive success and may shape the strength and targets of sexual selection. These processes likely contribute to the considerable within-population variation in social and mating behaviour observed in natural populations of *L. whitii* (Chapple and Keogh 2006; While, Uller, and Wapstra 2009a).

Resource structure and availability was not only important for dictating adult social and mating behaviour but also influenced the extent of prolonged associations between parents and offspring. My results show that resource availability and distribution influence the proportion of offspring that ultimately establish home ranges

overlapping that of their parents (chapters 3 and 4). Offspring given the chance to disperse into enclosures saturated with conspecifics were more likely to delay dispersal, suffered greater dispersal costs and were less successful in dispersal attempts compared to those given the opportunity to disperse into low saturation habitat. This resulted in greater levels of parent-offspring associations when available habitat was limited. Importantly, parent-offspring associations had measurable benefits for offspring in terms of growth and body condition, but I found no evidence that parents who tolerated offspring paid a cost.

Combined these results suggest some intriguing mechanisms mediating family dynamics that may provide the basis for future experimental work. An important distinction is whether the stability of family groups is mediated primarily by parental effects on offspring dispersal, interactions between siblings, or a combination of these influences. The lack of detectable costs to parents of tolerating offspring within their home range (chapter 3) and the increased costs to offspring of sharing the natal home site with their siblings (While GM unpublished data) suggests that dispersal may be mediated more so by offspring than by parents. Considerable aggression among neonate *L. whitii* siblings in the days following parturition supports this hypothesis (Botterill-James et al. in review). Antagonistic interactions among half sibs in litters of mixed paternity, as opposed to paternal-offspring conflict, could explain exclusion of extra-pair offspring from the home ranges of cuckolded males, as observed by While et al. (2009b). Indeed, my results suggest a combined influence of paternal tolerance and sibling competition on offspring dispersal, as sibling competition alone should not result in exclusion of extra-pair offspring from the home range of one parent and not the other. Importantly, the willingness of large offspring in good condition to disperse (chapter 4) also suggests that dispersal opportunities are actively pursued rather than resulting solely from expulsion by parents or competitively superior siblings.

Combined, these results suggest a relatively limited role for females in dictating family dynamics within this system. However, although it appears unlikely that females intervene directly to mediate conflicts they may influence conflicts indirectly via a number of different maternal effects. For example, the presence of facultative birthing asynchrony in *L. whitii* suggests that mothers may have a mechanism, the manipulation of birth spread, to alter the competitive environment of their offspring (While et al. 2007). This in turn could facilitate a social hierarchy that promotes dispersal of some

offspring (While and Wapstra 2007). We did not detect any effects of birth order on offspring growth, survival or dispersal behaviour in the current study (chapters 3 and 4). However the facultative nature of birthing asynchrony in this species suggests that adaptive explanations warrant further investigation (While and Wapstra 2007; While et al. 2007).

Independent of the exact feedbacks influencing family stability, common factors are likely to underpin how environmental conditions mediate these conflicts; namely, the terms identified by Hamilton's rule. These developments suggest a revision of the verbal model put forward by While et al. (2009b). Specifically, my work suggests that all family members may play a role in determining the composition and stability of family groups, but that conflicts will be contextualized by local ecological conditions. Rates of polyandry will be driven partly by habitat characteristics that promote inter-sex overlap (chapter 2), influencing the relatedness (r) among group members. Conflict between siblings may be as important as 'father-offspring' conflict in determining patterns of cooperation at the home refuge in the presence of polyandry; both social males and within-pair offspring will face reductions in relatedness (r) toward extra-pair offspring. While cuckolded males share no genes directly by decent with extra-pair offspring, the fine-grained genetic structure of *L. whitii* populations means that even extra-pair offspring will often confer an inclusive fitness benefit to cuckolded males (While et al. 2014). Given the lack of costs associated with tolerating offspring (chapter 3) small inclusive fitness benefits could potentially explain why extra-pair offspring are sometimes tolerated by cuckolded males, albeit rarely (While, Uller, and Wapstra 2009b). Considerable physical antagonism between siblings suggests that juvenile *L. whitii* undergo severe competition, increasing the costs (C) of cooperative behaviour, especially with extra-pair half sibs.

The magnitude of these costs will also be determined by local resource availability and population density (chapter 4), which define the costs of dispersal for offspring and the benefits (B) of being tolerated within the parental home range. Further work is required to quantify the contribution of conflict between different family members and to clarify the basis for variation in the motivation to disperse between individual offspring. However, the general patterns observed in our experiments demonstrate that habitat characteristics will be important for parameterising each term in Hamilton's rule and therefore the strength and stability of selection on family living.

Contributions to an understanding of social diversification across the Egernia group

The findings presented in section one provide several clear predictions concerning the influence of resource distribution and availability on social and mating behaviour in *Egernia*. First, where resource patches are small and uniformly distributed across the landscape small social groups centred around monogamous pair bonds are expected to form; where resources are heterogeneously distributed in clumped patches this should promote opportunities for social polygyny, potentially facilitating the accommodation of female-female tolerance and the emergence of larger multi-female social groups (chapter 2). Second, delayed dispersal and offspring tolerance should emerge, first and foremost, when habitat availability is limited (chapter 4). The key question moving forward is to evaluate how accurately these predictions reflect diversification in social organisation across the Egernia lineage and across taxa more generally.

Despite a growing number of studies investigating social organisation in Egernia species, there has been little progress toward understanding the processes that have led to diversification in social organisation across the group. This can perhaps be attributed to incongruent results between studies attempting to correlate variation in environmental characteristics with variation in social behaviour (e.g. Gardner et al. 2007; Michael et al. 2010). My results (chapters 2, 3 nd 4) provide the first clear experimental evidence of ecological context dependence in social and mating behaviour in an Egernia skink. These results mirror those found in studies of other vertebrates. For example, social organization and population dynamics in the bank vole, *Myodes glareolus,* are closely associated with the spatial distribution of food resources (Remy et al. 2013); males of the European bitterling, *Anodonta anatina*, that control clumped oviposition sites have greater opportunities for polygyny (Przybylski et al. 2009; Reichard 2004a;b) and the mating system of the European starling, *Sturnus vulgaris*, is the outcome of a behavioural feedback between male and female mating preferences and the distribution of suitable nest sites (Smith and Sandell 2005; Sandell and Smith 1997). The general coherence of my results with those from other vertebrate groups demonstrates the broad applicability of this work for understanding the causes of social living. However, more specifically, by showing that relatively minor manipulations in resource distribution and availability influence the composition and stability of social

groups in *L. whitii*, my results broadly support the hypothesis that environmental heterogeneity has been important in the evolutionary diversification of social organisation across the *Egernia* lineage (Duffield and Bull 2002; Chapple 2003; O'Connor and Shine 2003; Michael and Cunningham 2010).

The convergence of social systems among *Egernia* species that occupy distinct habitat types (e.g. *E. stokesii* and *E. frerei)* and, conversely, divergence in social organisation between those inhabiting seemingly similar habitats (e.g. *L. striata* and *L. kintorei*), demonstrates the inability of ecological characteristics alone to explain variation in social systems. Indeed, the physical arrangement of environments may dictate opportunities for social aggregation (Langkilde et al. 2007; Michael and Cunningham 2010) or the structure of social networks once they arise (Leu et al. 2016). However, the emergence and stability of social organization will ultimately depend on interactions between the environment, ecology and life history of the species under selection (Covas and Griesser 2007; Hatchwell 2009; Jetz and Rubenstein 2011). It is the interplay between all these factors that parameterize the cost-benefit trade-off of cooperative behaviour, including tolerance of kin and the expression of parental care toward offspring (Klug and Bonsall 2010; Bonsall and Klug 2011; Alonzo and Klug 2012; Wong et al. 2013).

Understanding broader patterns of squamate sociality

A key objective for ongoing research should be to integrate the work carried out in this thesis with comparative studies involving detailed fieldwork across a range of species and populations. Specifically, integrating data on social and life history traits with our predictions regarding the influence of local ecology on social and mating behaviour would provide a robust evaluation of the factors driving social diversity across the *Egernia* lineage. Although data sufficient to conduct such analyses are not yet available across the *Egernia* clade, we can still use inferences from our experimental work (chapters 2, 3 and 4) to inform hypothesis tests in broader comparative studies of social behaviour across the squamates. The literature review (chapter 5) presented in section two addressed the first basic requirement of this analysis; a firm understanding of the functional and taxonomic diversity of parental care in squamate reptiles. I show that lizards (and squamates in general) exhibit all but the most advanced forms of care found in other ectothermic taxa such as fish and amphibians, including nest

construction, egg brooding and defense, neonatal attendance and prolonged parent-offspring associations. Furthermore, the prevalence and taxonomic diversity of parental care uncovered by our review underlines squamates as a valuable and under-utilized system in studies of social evolution.

The correlation between viviparity and parent-offspring associations revealed by this literature search suggests that giving birth to live young should increase opportunities for interaction between parents and offspring at emergence, and may therefore promote a transition from solitary to group living (Queller 1994; Lion and van Baalen 2007). Indeed, we found that intergenerational social groupings have evolved considerably more often in viviparous species than in oviparous species, including oviparous species that attend their eggs (chapter 6). Live bearing was also associated with the evolutionary emergence of more complex social behaviour, with phylogenetic reconstructions indicating that viviparity has generally preceded the evolution of stable social groups in the squamates. These results highlight live bearing as an important precursor in the evolutionary origins of social organization in the squamates. Furthermore, they suggest that viviparity has provided a pre-adaptive context for the emergence and stabilization of more complex, kin-based social organization in the lineage. Combined with insights from our experimental work (chapters 2, 3 and 4) these findings provide exciting opportunites for future comparative studies exploring the evolutionary associaitons between ecological context, life history charcteristics and sociality in squamate reptiles.

Reflections

The global aim of my thesis was to connect processes occurring across levels of biological organization, with the intention of contributing to an understanding of the emergence, maintenance and diversification of social living. Although much work remains to be done, several important reflections have emerged from this work that allow us to think more broadly about research into the evolutionary ecology of social organization:

Reptiles have considerable heuristic merit in studies of social evolution. In particular, the occurrence of facultative social behaviour in a range of species with disparate phylogenetic affiliations, combined with the amenability of many species to

large scale experimental studies make reptiles an exceptional system to ask questions about the origins of vertebrate sociality. My work reveals that, despite these favourable traits, data on reptile sociality remains scarce. The primary challenge for future studies is therefore a lack of information regarding the extent of social behaviour for most species. I hope this study offers direction for targeted research on key taxonomic groups and encourages researchers to publish specific reports of social behaviour, or lack thereof, in squamate reptiles.

One potential reason for a lack of appreciation of sociality in reptiles is that reptiles generally lack overt social displays, thus social behaviour tends to remain relatively cryptic (Doody et al. 2012). However this thesis demonstrates that careful consideration of species ecology and life history can identify traits fundamental to the occurrence and maintenance of social behaviour. Future research should therefore target systems in which analogous conditions to those outlined above may have facilitated a convergent emergence of family living. My work identifies several exciting candidate groups for such studies: temperate pit vipers of North America, the cordylidae of sub-Saharan Africa and liolaemid iguanids of South America all display complex social behaviour including post-partum parental care and social aggregations of adults and juveniles. Available data also indicate variation in social traits between species, analogous to that observed in the Egernia (Greene et al. 2002; Halloy et al. 2007; Mouton 2011). Comparative studies incorporating information on ecology and life history would provide a powerful approach of evaluating whether common factors have been important in driving divergence in social traits among these disparate groups. The first step toward realizing these analyses is detailed fieldwork characterizing variation in social behaviour across a broader range of species.

Sociality is a product of many different aspects of the biology of a species, but simple social organization emerges readily when the right ecological conditions are met. The initial emergence of social behaviour will be driven primarily by factors that increase the likelihood of social contact between conspecifics. For example, any factor that promotes delayed dispersal among offspring will enhance opportunities for social interaction between parents and offspring, and therefore the potential for selection to act on beneficial interactions when they do occur. Importantly, my work suggests that the persistence of these associations does not require any specific benefits, as levels of social interaction may be driven largely by variation in ecological

conditions that dictate how individuals use their environment (Chapters 3 and 4). However, as long as the costs to parents of tolerating offspring are low enough, benefits conferred to offspring of remaining within the natal territory can easily yield inclusive fitness benefits (Klug et al. 2012). This demonstrates how easily the initial steps towards social organisation can be achieved, and the potential for minor alterations in social interactions to provide a pre-adaptive context for the evolution of more complex forms of social behaviour. Indeed, in the presence of ecological conditions that reduce the costs of intra-familial resource competition; life-history characteristics conducive to delayed dispersal and parental investment; and phylogenetic affiliations that do not constrain the evolution of behavioural care traits, any factors that promote repeated social contact between generations may set the stage for the evolution of more complex forms of parental care, cooperative behaviour and ultimately, kin-based social organization.

Furthermore, studies attempting to understand the occurrence of social organization typically account for the maintenance of social behaviour as a cost-benefit trade-off in the face of considerable costs associated with cheaters and defectors (Nowak 2006; West et al. 2007; Strassmann and Queller 2011; Ho et al. 2013), and conceive of the evolutionary impetus for transitions in social complexity in terms of the mutual or inclusive fitness benefits of cooperation (Boomsma 2009; Cornwallis et al. 2010). However focusing solely on the adaptive benefits of cooperation may constrain our ability to understand factors driving the initial emergence of social behaviour; such a perspective overlooks the conditions that originally promote or enable social contact between individuals (Uller and Helanterä 2014). This thesis demonstrates how a mechanistic perspective, emphasizing the importance of conditions that enhance opportunities for the emergence of social traits, may provide elegant solutions for investigating the evolutionary origins of social behaviour.

Establishing meaningful connections between pattern and process is vital in studies of evolutionary ecology. If we want to understand how sociality emerges and diversifies over evolutionary time we need an integrated approach that first identifies the causes and consequences of variation in social behaviour in ecological settings, and then translates this understanding into a set of general principles that adequately describe the variation we see across species. By integrating prior knowledge from natural population observations with experimental field studies and comparative

analyses, my thesis attempts to bridge the gap between these empirical approaches. I show that an understanding of species biology can indeed be scaled up to inform studies examining patterns of diversity at higher levels of biological organisation. Importantly, the most comprehensive and informative insights will be gained by focusing research efforts on taxonomic groups that allow us to both form clear predictions of phylogenetic patterns in trait distribution, as well as explicitly test hypotheses of functional links between traits. Only by connecting pattern and process at each stage will we be able to close the loop on a holistic understanding of social evolution.

Concluding remarks

This thesis emphasizes the importance of ecological heterogeneity in influencing patterns of social and mating behaviour. It suggests that relatively minor changes in ecological conditions can alter the evolutionary trajectory of populations and contribute toward transitions in social complexity. In particular, my work highlights the importance of traits, both biotic and abiotic, that promote ongoing social contact between conspecifics, as stable social associations are the first step toward the emergence of more complex social behaviour. Looking forward, targeted comparative studies incorporating climatic, environmental and life-history data will continue to refine our understanding of social evolution. The facultative nature of many social traits in reptiles makes them uniquely poised for studies investigating the evolutionary origins of social behaviour, providing opportunities to address questions that fall outside the epistemic limits of more traditional avian and mammalian systems. Continued work on reptile sociality will not only fill gaps in our understanding of social behaviour in a major vertebrate order, but may also provide new insights into the factors responsible for the emergence, maintenance and diversification of social behaviour itself.

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Appendix I

Supplementary Information for chapter two: Resource Distribution Mediates Social and Mating Behavior in a Family Living Lizard

Overdispersion

In order to account for overdispersion in a Poisson GLMM, it is often sufficient to include an individual level random variable, effectively translating a Poisson model to a lognormal-Poisson model. In the case of our data this means simply including a random effect of ID nested within our other random effect, enclosure (yielding: enclosure/ID).

Discussed here by developer of the lme4 package, Ben Bolker:
https://stat.ethz.ch/pipermail/r-sig-mixed-models/2010q4/004761.htm

Justification for methods of permanently marking lizards

(Based on Uller, T. 2010. *Lizards in Biological Research. Module 3, Licensee Training Course,* Veterinary Services, University of Oxford)

NOTE: These recommendations do not apply to lizards with enlarged toe pads used for climbing (e.g., geckos, some Anolis species)

Reliable marking of individuals is of utmost importance in field studies, without which researchers would be unable to address many fundamental questions in ecology and evolution. By far the most common method in lizard research, both historically and currently, is removal of one or two joints on up to two digits per foot using surgical scissors (Woodbury et al. 1956; Ferner 2007; Ferner and Plummer 2016). Individual identification using coloration patterns is rarely possible and not feasible in long-term projects involving large numbers of animals (Gent and Gibson 2003; Ferner 2007). Specifically, for *L.* whitii, it is impossible to distinguish individuals based solely on colour

markings. Furthermore, we have experimented with additional methods of temporary marking, including paint. Unfortunately, as *L. whitii* is a burrowing lizard, paint weathers very quickly and only allows unique identification for a short-time period before re-application is necessary. This would require very frequent re-capture events, which produce comparable stress responses in lizards to the toe clipping procedure itself (see below). This means that when conducting experiments on captive populations of numerous individuals, paint markings that degrade very quickly can easily result in individuals being falsely identified and may compromise the aims of the study. We do use industry grade cloth stickers (the most reliable form of non-permanent marking based on over 40 years of combined experience working with reptiles among authors) for easy identification to reduce our rate of re-capture, but these are also non-permanent and rely on the permanent identification provided by toe clipping to accurately keep track of individual ID.

The development of microchip technology now also allows an alternative method of permanent marking: pit-tagging of moderately sized lizards (Ferner 2007; Ferner and Plummer 2016). However, there are a number of reasons to prefer removal of digits over pit-tagging of small to moderate-sized lizards (see Gent and Gibson 2003; Ferner 2007 for a detailed discussion). First, the most comprehensive study to date to address stress in relation to marking methods in lizards (This study was conducted on another Australian scincid, comparable to the species used in our study in terms of size and ecology; Langkilde & Shine 2006) found that digit removal only resulted in a small and short-term increase of stress levels comparable to that of mild handling and exposure to predator scent. In contrast, the implantation of pit tags resulted in significantly higher stress levels (but still not higher than naturally observed increases stress levels resulting from predation attempt). Second, there is no evidence that digit removal has a lasting effect on physiological stress levels (Langkilde and Shine 2006). The data from Langkilde & Shine is in line with the observation that pain associated with digit removal occurs at a considerably lower level than does removal of, for example, ectoparasites (ticks: Uller and Olsson 2003; T Uller; GM While; E Wapstra pers obs based on handling thousands of lizards). Third, the handling time for digit removal is much shorter than for pit-tagging, which reduces overall stress associated with capture and marking. Fourth, several studies have documented that there are no negative effects of digit removal on several important welfare and fitness components, including corticosterone response, sprint speed, growth,

and survival in the field (Dodd 1993; Hudson 1996; Paulissen and Meyer 2000; Borges-Landáez and Shine 2003; reviewed in Gent and Gibson 2003; Ferner 2007; Perry et al. 2011). Fifth, micro-chip technology is simply not possible for small lizards and particularly juveniles because the size of the microchips are above those required for minimum chip to body size ratios (Ferner and Plummer 2016). Finally, a recent review summarizing this evidence found toe clipping to meet both legal and ethical expectations of scientific research involving reptiles (Perry et al. 2011).

Concerning tail clipping for genetic analyses, unfortunately, toe joints removed during clipping are typically insufficient for DNA extraction in *L. whitii*, as the toe itself contains very little workable tissue. This necessitates an additional tissue sample for genetic analyses. Tail clipping is a standard method of obtaining genetic material in current reptile research and was also approved for use in this project after consideration by the University of Tasmania animal ethics committee. Furthermore, *L. whitii* displays caudal autotomy (voluntary tail dropping) when threatened, therefore partial tail loss is a relatively common natural occurrence. Indeed, natural population studies show that 30% of animals are captured with some portion of their tail missing or with evidence of past autotomy (GM While, unpublished data). Species that display this trait also possess physiological and morphological adaptations to reduce its impact on homeostasis (Clause and Capaldi 2006; Gilbert et al. 2013). We remove only the terminal 5mm of tail tissue for genetic analyses. This does not appear to cause any distress to animal subjects and grows back within several weeks, before animals are released back into natural populations.

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Figure A1.1. Animal enclosure compound used to conduct experiment. Photo shows the two different enclosure treatments (center left).

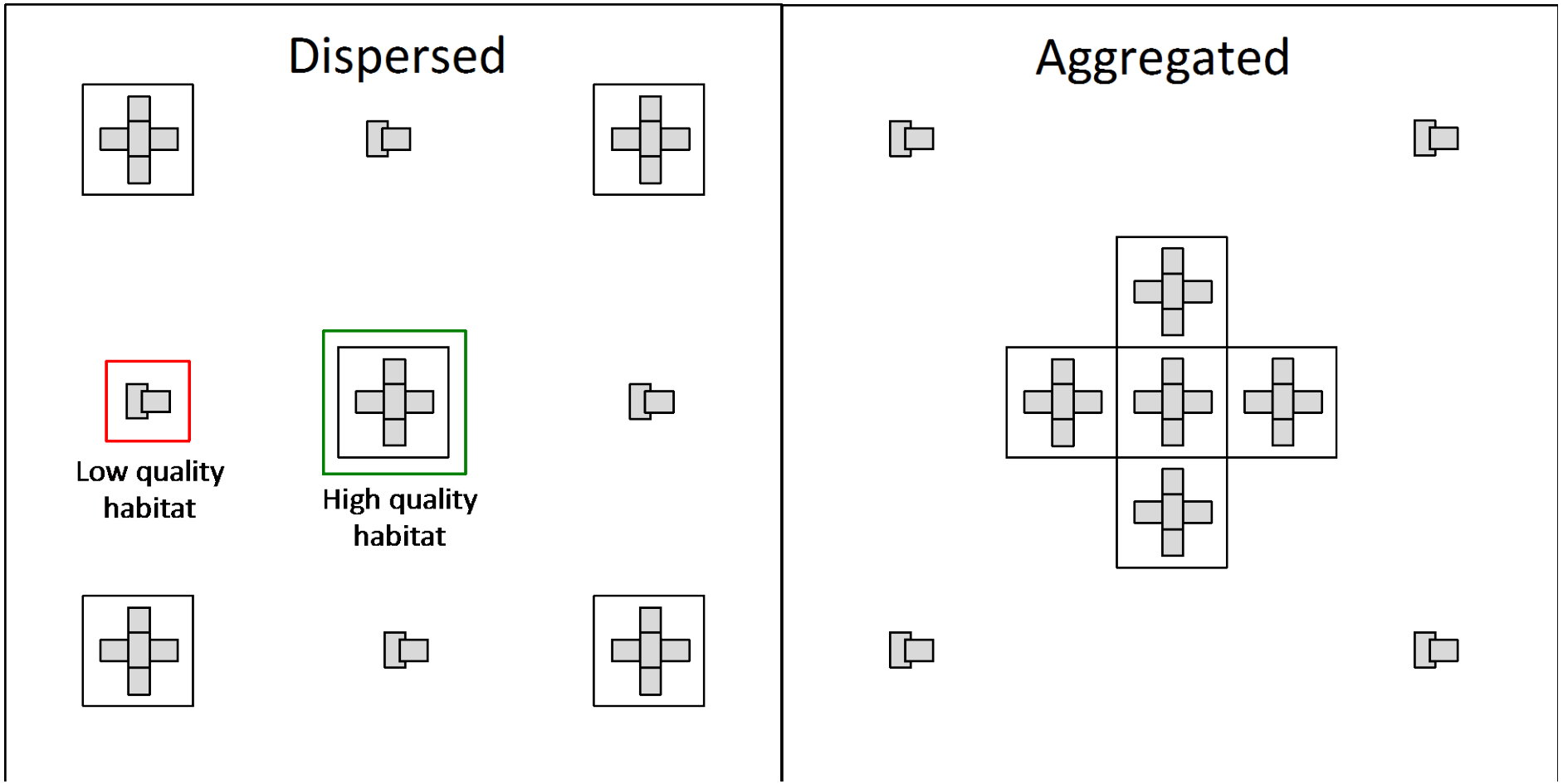


Figure A1.2. Schematic showing the design of the two enclosure treatment

Figure A1.3. Cloth stickers used for rapid identification during enclosure observations

Table A1.1. Results of a Gaussian GLM confirming no difference in SVL or Mass of either sex between treatment groups. Significant terms shown in bold.

Predictor	SVL	Mass
Treatment	F1,156 = 0.01, P = 0.904	F1,156 = 0.34, P = 0.563
Sex	**F1,156 = 7.74, P = 0.006**	**F1,156 = 4.22, P = 0.042**
Treatment:Sex	F1,156 = 0.54, P = 0.462	F1,156 = 1.4 P = 0.239

Table A1.2. Mean (%±SE) kernel home range overlap of different overlap classes. Restricted to individuals with >8 observations.

Treatment	male-female	social partners	male-male	female-female
Aggregated	17.5±2.2	56.3±3.8	8±2.5	7.4±3.6
Dispersed	11.9±1.9	55.7±4.9	6.7±4.4	4.4±6.4

Table A1.3. Results of LMMs and GLMMs for male and female home range characteristics. Significant terms shown in bold. Marginally non-significant terms indicated by an asterisk.

Predictor	Home Range Size (50%)	Inter-sex Overlap	Intra-sex Overlap
Treatment	**F1,13.8 = 12.19, P = 0.003**	**χ2 = 8.11, P = 0.004**	χ2 = 3.8, P = 0.052 *
Sex	F1,125.9 = 0.91, P = 0.341	χ2 = 0.86, P = 0.355	**χ2 = 7.7, P = 0.005**
SVL	F1,136.2 = 1.86, P = 0.18	χ2 = 1.31, P = 0.252	χ2 = 0.69, P = 0.405
Treatment:Sex	F1,126.16 = 0.001, P = 0.97	χ2 = 0.02, P = 0.893	χ2 = 0.17, P = 0.676
Body Condition (covariate)	F1,136.7 = 2.65, P = 0.11	χ2 = 0.40, P = 0.526	χ2 = 0.64, P = 0.424

Table A1.4. Results of LMMs testing the effect of occupying high quality habitat on male and female recapture body condition. Significant terms shown in bold.

Sex	Predictor	Recapture Body Condition
Male	Habitat Quality	**F1,60.8 = 6.85, P = 0.011**
	Treatment	F1,16.1 = 3.04, P = 0.1
	SVL	F1,63 = 0.57, P = 0.542
	Body Condition (covariate)	**F1,64.5 = 5.52, P = 0.022**
Female	Habitat Quality	**F1,28.7 = 6.2, P = 0.019**
	Treatment	F1,16.2 = 0.31, P = 0.585
	SVL	F1,24.5 = 0.55, P = 0.464
	Clutch Mass (covariate)	**F1,28.1 = 8.31, P = 0.007**
	Body Condition (covariate)	F1,62.8 = 0.17, P = 0.68

Table A1.5. Results of a binomial GLMM testing the effect of treatment on the probability of occupying HQ habitat. Significant terms shown in bold.

Predictor	Occupied HQ Habitat
Sex	$\chi2 = 0.13$, P = 0.714
Treatment	**$\chi2 = 11.61$, P < 0.001**
SVL	$\chi2 = 0.26$, P = 0.61
Body Condition (covariate)	$\chi2 = 0.7$, P = 0.403
Sex:Treatment	$\chi2 = 0.25$, P = 0.616

Table A1.6. Results of Poisson GLMMs of male reproductive success. Columns display the results of two separate models run on different subsets of the data. Significant terms shown in bold. Marginally non-significant terms indicated by an asterisk.

Predictor	RS (males in social groups)	RS (all males)
Treatment	$\chi2 = 0.27$, P = 0.604	$\chi2 = 0.72$, P = 0.397
Polygyny	$\chi2 = 3.51$, P = 0.061	-
Social	-	**$\chi2 = 13.59$, P < 0.001**
SVL	$\chi2 = 1.47$, P = 0.223	**$\chi2 = 7.34$, P = 0.007**
Head Legth	-	$\chi2 = 3.24$, P = 0.072*
Treatment:Polygyny	$\chi2 = 2.41$, P = 0.121	-
Treatment:SVL	-	$\chi2 = 0.35$, P = 0.550
Treatment:Head Length	-	$\chi2 = 0.95$, P = 0.328
Body Condition (covariate)	**$\chi2 = 16.96$, P < 0.001**	**$\chi2 = 18.20$, P < 0.001**

Table A1.7. Results of a Poisson GLMM testing for a difference in Bateman gradient between treatments. Significant terms shown in bold.

Predictor	RS (all males)
Treatment	**χ2 = 7.97, P = 0.005**
No. of partners	**χ2 = 120.29, P < 0.001**
Treatment:No. of partners	**χ2 = 17.32, P < 0.001**

Table A1.8. Results of a Binomial GLMM testing for a difference between treatments in the probability that social groups are polygynous. Significant terms shown in bold.

Predictor	Polygyny
Treatment	**χ2 = 5.17, P = 0.023**
SVL	χ2 = 0.35, P = 0.56
Body Condition (covariate)	χ2 = 0.73, P = 0.39

Table A1.9. Results of a Binomial GLMM testing for a difference in the propensity of males to form social groups. Significant terms shown in bold.

Predictor	Social
Treatment	χ2 = 1.41, P = 0.24
Inter-sex overlap	**χ2 = 21.46, P < 0.0001**
SVL	χ2 = 1.22, P = 0.27
Body Condition (covariate)	χ2 = 1.85, P = 0.17

Table A1.10. Results of a Binomial GLMM testing for the effects of treatment and phenotypic traits on whether females produced a clutch. Significant terms shown in bold. Marginally non-significant terms indicated by an asterisk.

Predictor	Reproduced
Treatment	χ2 = 0.16, P = 0.69
SVL	**χ2 = 7.92, P = 0.005**
Body Condition	χ2 = 3.44, P = 0.06*

Table A1.11. Results of a Poisson GLM testing for the effect of treatment and phenotypic traits on clutch size among reproducing females. Significant terms shown in bold.

Predictor	Clutch Size
Treatment	χ2 = 0.16, P = 0.69
SVL	χ2 = 0.65, P = 0.42
Body Condition	χ2 = 0.67, P = 0.41

Table A1.12. Results of a Binomial GLMM testing the effect of treatment on rates of EPP. Marginally non-significant terms indicated with an asterisk.

Predictor	EPP
treatment	χ2 = 0.007, P = 0.934
inter-sex overlap	χ2 = 3.73, P = 0.054*
SVL	χ2 = 1.35, P = 0.7133
Body Condition (covariate)	χ2 = 0.04, P = 0.835

Appendix II

Supplementary Information for chapter three: Habitat Structure Influences Parent-Offspring Association in a Social Lizard

Figure A2.1. Photos of the enclosures used in the experiment. On the left, a dispersed treatment is shown in the foreground, and an aggregated treatment is shown immediately behind. On the right is a close up of a high quality habitat, with recently released lizards on the top Besser block.

Figure A2.2. Semi-permanently marked juvenile *L. whitii*. The yellow bee tag indicates that this offspring was born to mother number four in the enclosure, and the positioning of the tag indicates that it was the first offspring of the litter.

Appendix III

Supplementary Information for chapter four: Habitat Saturation Promotes delayed Dispersal in a Social Reptile

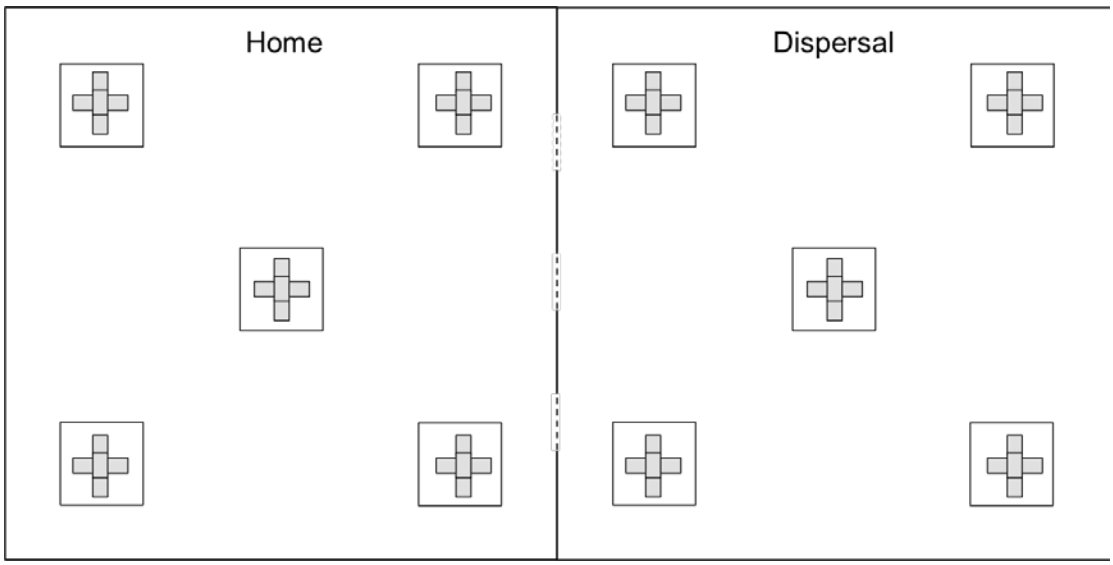


Figure A3.1. Diagrammatic representation of an enclosure pair showing arrangement of habitat patches.

Table A3.1. The effect of treatment, exploration, SVL, body condition and positive associations (PA) on different fitness related traits. Significant terms shown in bold. Marginally non-significant results indicated by an asterisk. Dashes indicate terms that were not included in the specified model.

Variable	Recapture condition	Growth	Survival
Treatment	F1,11.5 = 0.919, P = 0.357	F1,10.2 = 0.023, P = 0.883	**χ2 = 4.647, P = 0.031**
Explore	**F1,33.4 = 5.578, P = 0.024**	F1,34.3 = 0.649, P = 0.426	χ2 = 1.405, P = 0.236
PA	**F1,32.7 = 5.772, P = 0.022**	F1,33.4 = 0.154, P = 0.698	χ2 = 0.393, P = 0.531
Treatment:Explore	**F1,31.2 = 8.972, P = 0.005**	F1,33.2 = 0.001, P = 0.998	**χ2 = 4.673, P = 0.031**
SVL (covariate)	F1,28.4 = 0.357, P = 0.555	F1,34.8 = 0.715, P = 0.403	χ2 = 3.765, P = 0.052 *
Condition (covariate)	**F1,33.3 = 9.341, P = 0.004**	-	χ2 = 1.007, P = 0.316
Age (covariate)	**F1,33.4 = 4.9, P = 0.034**	-	χ2 = 0.239, P = 0.625
Duration (covariate)	-	**F1,3.6 = 18.211, P = 0.016**	-

Table A3.2. Same models as featured in table A3.1 but with Delayed Dispersal (highlighted) rather than PA included as a predictor to test for benefits of philopatry. Significant terms shown in bold. Marginally non-significant results indicated by an asterisk. Dashes indicate terms that were not included in the specified model.

Variable	Recapture condition	Growth	Survival
Treatment	F1,10.5 = 0.375, P = 0.554	F1,8.1 = 0.042, P = 0.842	**χ2 = 3.985, P = 0.046**
Explore	**F1,33.4 = 7.207, P = 0.011**	F1,34.4 = 0.509, P = 0.480	χ2 = 1.506, P = 0.220
Delayed Dispersal	F1,28.7 = 0.731, P = 0.400	F1,18.4 = 1.052, P = 0.318	χ2 = 0.895, P = 0.344
Treatment:Explore	**F1,33.0 = 7.044, P = 0.012**	F1,34.9 = 0.0062, P = 0.805	**χ2 = 4.883, P = 0.027**
SVL (covariate)	F1,29.8 = 0.297, P = 0.590	F1,34.8 = 0.629, P = 0.433	χ2 = 2.844, P = 0.092 *
Condition (covariate)	**F1,32.8 = 8.479, P = 0.006**	-	χ2 = 1.228, P = 0.268
Age (covariate)	F1,33.7 = 3.921, P = 0.056*	-	χ2 = 0.280, P = 0.596
Duration (covariate)	-	**F1,2.8 = 16.548, P = 0.032**	-

Appendix IV

Supplemental Experimental Procedures for chapter six: Correlated Evolution Between Viviparity and Social Grouping in Squamate Reptiles

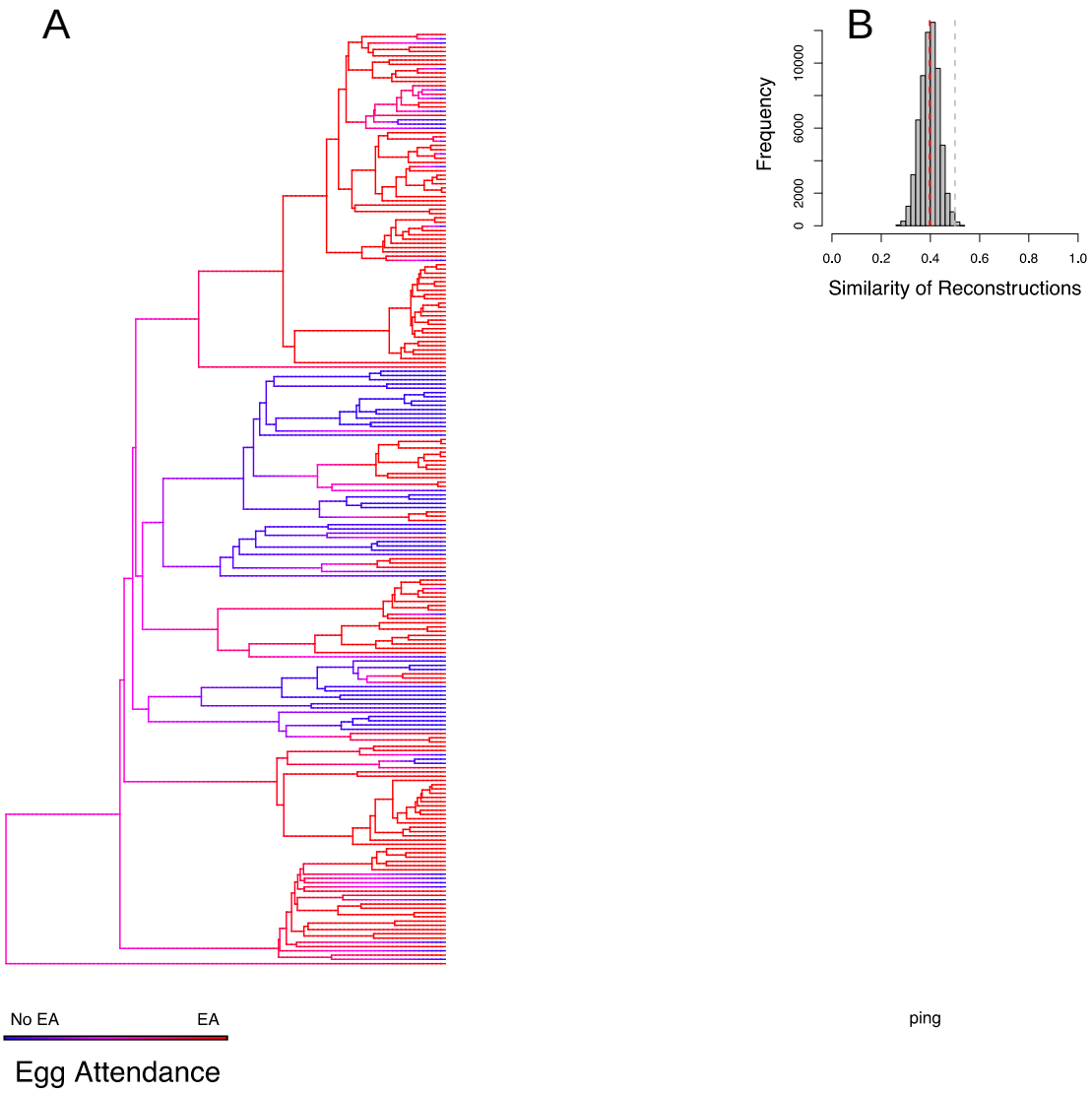


Figure A4.1. A: **No evidence for correlated evolution between egg attendance and social grouping among oviparous squamate reptiles** based on ancestral state reconstruction by stochastic character mapping. Phylogeny restricted to oviparous species in the conservative data set (n = 219, see below). Branch colours represent posterior probability densities of edge states based on 1000 stochastic character maps of each reconstruction. **B**: Distribution of similarity scores between stochastic character map sets (n = 1000) based on separate ancestral character state reconstructions of egg attendance and social grouping. The grey line represents the null expectation of similarity between map sets if the two traits being compared show no evolutionarily correlation during reconstruction. The red line represents the mean similarity between map sets based on our reconstructions.

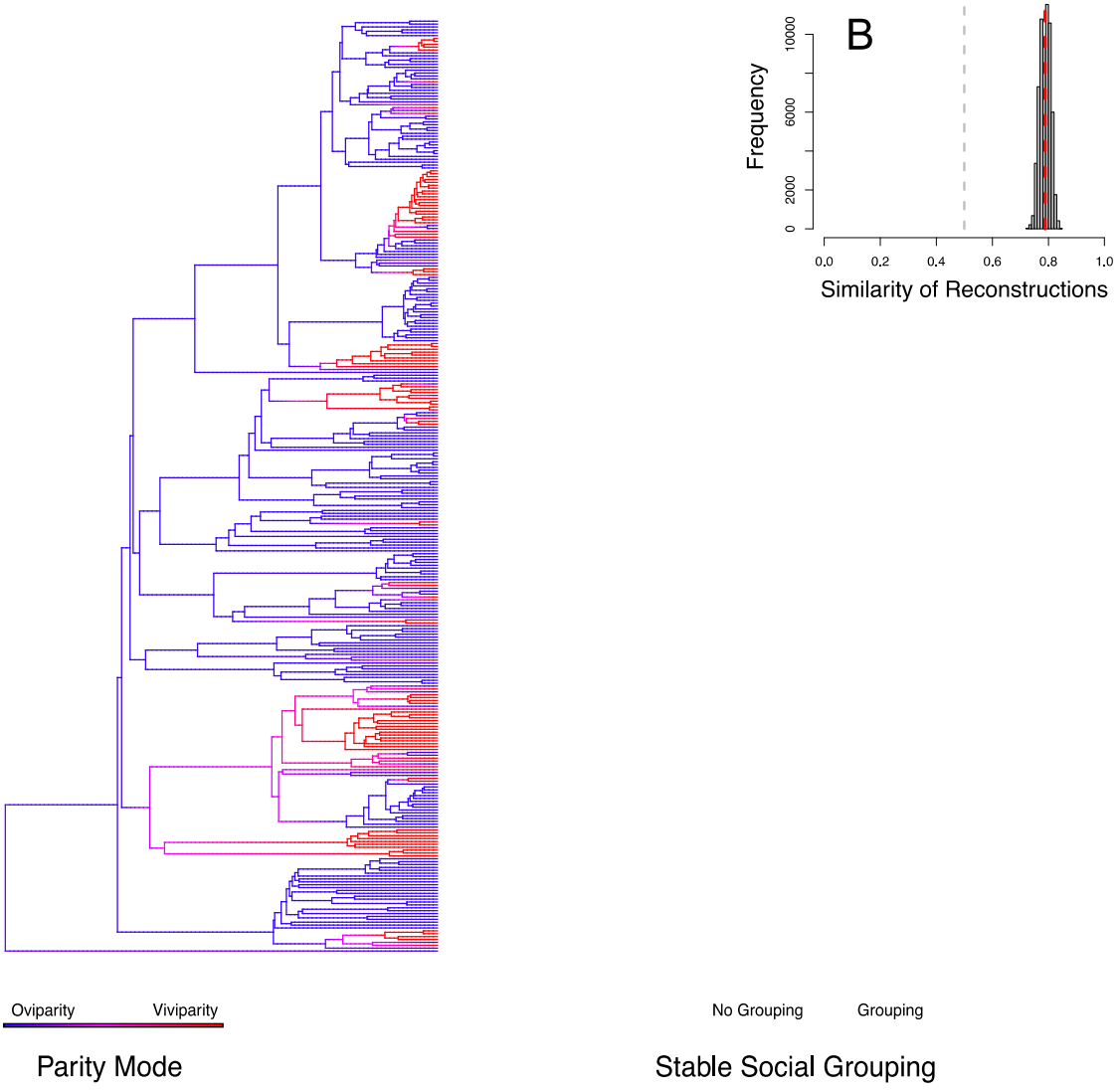
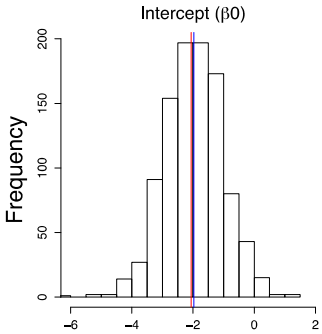


Figure A4.2. A: **Correlated evolution of viviparity and stable social grouping among squamate reptiles** based on ancestral state reconstruction by stochastic character mapping. Phylogeny restricted to species in the conservative data set (n = 324, see below). Branch colours represent posterior probability densities of edge states based on 1000 stochastic character maps of each reconstruction. **B**: Distribution of similarity scores between stochastic character map sets (n = 1000) based on separate ancestral character state reconstructions of egg attendance and social grouping. The grey line represents the null expectation of similarity between map sets if the two traits being compared show no evolutionarily correlation during reconstruction. The red line represents the mean similarity between map sets based on our reconstructions.



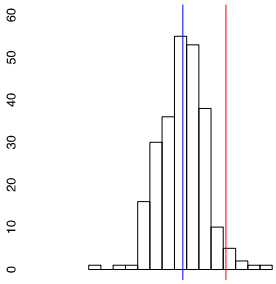


Figure A4.3. Posterior distributions of parameter estimates (β0, β1, s2) from binary phylogenetic generalized linear mixed models of the relationship between parity mode and social grouping from simulated data. The blue lines represent the mean of the posterior distribution of each parameter estimate from 1000 (**A**, **B**) or 250 (**C**, **D**) model simulations. The red lines represent the original parameter estimates from the real data models (Table 1). **A**: Conservative zeros data set, parity model. **B**: Conservative zeros data set, egg attendance model. **C**: Relaxed zeros data set, parity model. **D**: Relaxed zeros data set, egg attendance model.

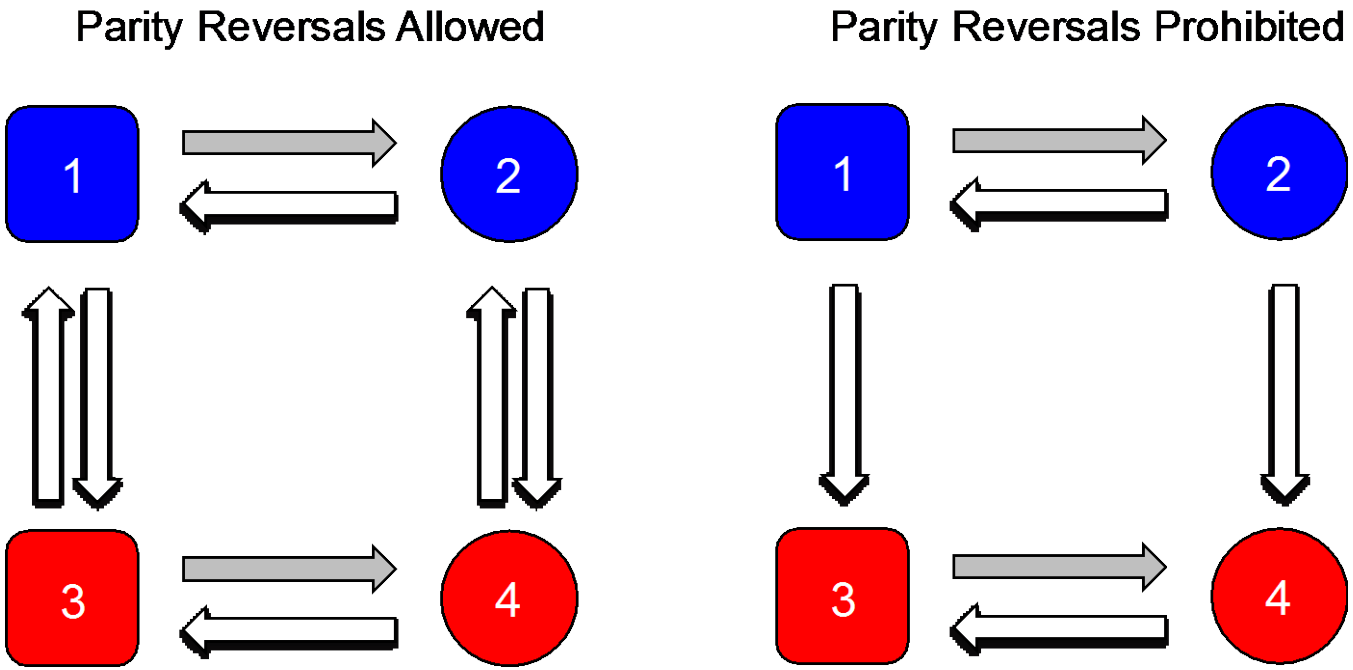


Figure A4.4. Structure of the two MuSSE models used to estimate rates of transition toward social grouping (SG) from a background of each parity mode. Blue represents oviparity, red represents viviparity; Squares are social grouping absent, circles are social grouping present. Simultaneous double transitions are prohibited (i.e. no diagonal arrows). Numbers correspond to the four possible states in each model (1: oviparous, no SG; 2: oviparous, SG; 3: viviparous, no SG; 4: viviparous, SG; also see Table S1). Grey arrows represent the transition rates that were constrained to be equal during likelihood ratio tests of the difference in transition rate towards SG between parity modes (i.e. 'Test' models in Table A4.1).

Table A4.1. Parameter estimates and likelihoods for alternative models constructed in the MuSSE framework. State dependent speciation (**λ**) and extinction (**μ**) rates are allowed for all states (1: oviparous, no SG; 2: oviparous, SG; 3: viviparous, no SG; 4: viviparous, SG). Light grey cells indicate transition rates that have been fixed to be equal. Black cells indicate transition rates that have been constrained to zero (parameters representing simultaneous double transitions (e.g. q14) constrained to zero but not shown). In test models, rates of transition toward social grouping from a background of oviparity and viviparity have been constrained to be equal to perform a likelihood ratio test comparing differences in model fit when transition rates toward social grouping from each parity mode are allowed to vary. Transition rates toward social grouping are always higher from a background of viviparity (q34) than oviparity (q12) whether parity reversal are allowed or prohibited. Significant P values in bold.

Parity reversals	Model	Df	ΔAIC	X^2	P	λ1	λ2	λ3	λ4	μ1	μ2	μ3	μ4	q12	q13	q21	q24	q31	q34	q42	q43
Conservative zeros data set, complete cases analysis (n = 324)																					
Prohibited	Fit	14				0.43	0.055	0.881	0.004	0.37	<0.001	0.749	<0.001	0.002	0.006	0.028	<0.001		0.127		0.025
	Test	13	34.3	36.3	**<0.001**	0.43	0.052	0.638	0.39	0.366	<0.001	0.62	0.29	0.004	0.009	0.027	0.002		0.004		0.018
Allowed	Fit	16				0.428	0.055	0.885	0.004	0.369	<0.001	0.753	<0.001	0.002	0.006	0.028	<0.001	<0.001	0.127	<0.001	0.025
	Test	15	16.7	18.7	**<0.001**	0.479	0.048	0.525	0.409	0.435	<0.001	0.468	0.324	0.005	0.003	0.031	0.003	0.02	0.005	<0.001	0.014
Relaxed zeros data set, complete cases analysis (n = 1210)																					
Prohibited	Fit	14				0.147	0.035	0.207	0.061	0.06	<0.001	0.084	<0.001	<0.001	0.001	0.014	0.002		0.026		0.009
	Test	13	14	16.5	**<0.001**	0.151	0.037	0.275	0.129	0.065	<0.001	0.213	0.05	0.001	0.002	0.015	0.004		0.001		0.016
Allowed	Fit	16				0.138	0.042	0.273	0.076	0.045	<0.001	0.194	0.051	<0.001	0.001	0.021	0.001	0.008	0.019	0.004	0.006
	Test	15	10	12.1	**<0.001**	0.148	0.039	0.404	0.128	0.062	<0.001	0.362	0.083	0.001	<0.001	0.017	0.002	0.016	0.001	0.003	0.018
Conservative zeros data set, all species in phylogeny (n = 3952, response of species with unknown state of social grouping (n = 3628) estimated by model)																					
Prohibited	Fit	14				0.094	0.024	0.206	0.014	<0.001	<0.001	<0.001	0.01	0.033	0.009	0.01	<0.001		0.174		0.001
	Test	13	156	158	**<0.001**	0.122	0.033	0.009	0.074	<0.001	<0.001	<0.001	<0.001	0.016	0.043	0.01	<0.001		0.016		0.042
Allowed	Fit	16				0.061	<0.001	0.188	0.045	<0.001	<0.001	<0.001	<0.001	0.021	<0.001	0.005	<0.001	0.107	0.033	0.009	0.1
	Test	15	4	5.9	**0.015**	0.058	<0.001	0.189	0.051	<0.001	0.003	<0.001	<0.001	0.022	0.003	0.005	0.002	0.122	0.022	<0.001	0.009
Relaxed zeros data set, all species in phylogeny (n = 3952, response of species with unknown state of social grouping (n = 2742) estimated by model)																					
Prohibited	Fit	14				0.074	0.051	0.171	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.007	0.01		0.093		0.004
	Test	13	153	155	**<0.001**	0.076	0.042	0.152	0.078	<0.001	<0.001	<0.001	<0.001	0.001	0.001	0.006	0.001		0.001		0.005
Allowed	Fit	16				0.06	0.088	0.172	0.012	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	0.065	0.066	0.036	0.011	0.005
	Test	15	17	19.3	**<0.001**	0.047	0.03	0.186	0.095	<0.001	<0.001	<0.001	<0.001	0.001	0.001	0.004	0.007	0.148	0.001	0.011	0.037

Table A4.2. Number of species from different squamate families displaying each form of social grouping. Numbers in parentheses represent the subset of all species within a category that are included in the phylogeny of Pyron et al. [19] and therefore species included in analyses.

	Social Grouping	Stable Social Grouping
Amphisbaenia		
Trogonophidae	1	0
Amphisbaenidae	1 (0)	0
Total	**2 (1)**	**0**
Sauria		
Agamidae	7	1
Anguidae	3 (2)	0
Carphodactylidae	1	0
Cordylidae	7 (6)	2
Diplodactylidae	4	1
Geckkonidae	4	0
Lacertidae	1	0
Leiocephalidae	1	1
Liolaemidae	6 (5)	1
Phrynosomatidae	1	1
Phyllodactylidae	1	0
Scincidae	31 (25)	11 (8)
Tropiduridae	1	1
Xantusiidae	2	2
Xenosauridae	3 (1)	1 (0)
Total	**71 (62)**	**22 (18)**
Serpentes		
Boidae	1	0
Colubridae	1	0
Elapidae	1 (0)	0
Pythonidae	2	0
Typhlopidae	1 (0)	0
Viperidae	17	1
Total	**23 (21)**	**1**
Grand Total	**98 (84)**	**23 (19)**

Table A4.3. Number of species from different squamate families displaying each parental care trait listed in Table A4.4. Many species display multiple forms of care, therefore the final column shows the total number of species from each family reported to show any form of care. Numbers in parentheses represent the subset of all species within a category that are included in the phylogeny of Pyron et al. [19] and therefore species included in analyses. Where no parentheses are shown all species are included in the phylogeny.

	Nesting	Egg Manipulation	Defence	Assist Neonate	Parent-offspring Association	Number of Species
Amphisbaenia						
Trogonophidae	0	0	0	0	1	1
Amphisbaenidae	0	0	0	0	1 (0)	1 (0)
Total	**0**	**0**	**0**	**0**	**2 (1)**	**2 (1)**
Sauria						
Agamidae	6	0	5 (4)	1	7	11 (10)
Anguidae	3	14 (8)	5 (3)	3	2 (1)	19 (13)
Cordylidae	1	0	0	1	4 (3)	4 (3)
Crotaphytidae	0	0	1	0	0	1
Diplodactylidae	0	0	3	0	3	4
Gekkonidae	1	9	11	2	2	12
Iguanidae	12	0	12	0	0	14
Lacertidae	2	2	1	0	1	5
Leiocephalidae	0	0	0	0	1	1
Liolaemidae	1	0	2	1	6 (5)	7 (6)
Phrynosomatidae	2 (1)	0	0	0	2	4 (3)
Phyllodactylidae	4	3	3	0	0	5
Scincidae	17 (14)	29 (23)	17 (13)	11 (9)	26 (21)	53 (40)
Sphaerodactylidae	1	0	1	0	0	2
Teiidae	2	2	2	0	0	3
Tropiduridae	3	0	3	0	1	4
Varanidae	9	1	6	2	0	8
Xantusiidae	0	0	0	2	2	3
Xenosauridae	0	0	0	0	3 (1)	3 (1)
Total	**64 (60)**	**60 (48)**	**72 (65)**	**23 (21)**	**60 (50)**	**168 (143)**
Serpentes						
Boidae	0	9	2	4	1	9
Calabariidae	0	1	0	0	0	1
Colubridae	10 (8)	18 (16)	5	0	0	23 (21)
Elapidae	5	17 (14)	8 (7)	0	0	17 (14)
Lamprophiidae	1	4 (3)	0	0	0	4 (3)
Leptotyphlopidae	1	1	0	0	0	1
Pythonidae	3	27 (23)	10 (8)	0	2	27 (23)
Viperidae	1	14 (12)	16 (15)	0	17	35 (33)
Total	**21 (19)**	**91 (79)**	**41 (37)**	**4**	**20**	**117 (105)**
Grand Total	**85 (79)**	**151 (127)**	**113 (102)**	**27 (25)**	**82 (71)**	**287 (249)**

Supplemental Experimental Procedures

Data collection

We searched the literature for reports of parental care and social association in squamate reptiles, including both pre- and post-partum care behaviours. We began our search by extracting all relevant data from the most comprehensive review of parental behaviour in squamate reptiles to date [1]. We augmented this data, excluding any reports that were highlighted in the text as uncertain, then extended upon it by adding new reports of parental care and updating previous reports based on current literature. Thus, we used ISI Web of Science to search for all articles from 2003 to the present using the search terms 'reptile', 'lizard', 'snake', 'amphisbaenia' and 'squamate', combined with 'nest*', 'guard*', 'defense', 'brood*', 'provision*', 'parental', 'care', 'tolerance' and 'oophagy'. We combined results from these searches with those from a recent review of social aggregation in squamate reptiles [2], including only those species reported to display aggregations containing both adults and juveniles. Wherever possible we accessed the primarily literature cited in these reviews to confirm reports of these associations, but relied on the interpretation of these authors when we could not access primarily sources.

Quantifying the functional and taxonomic diversity of care

In order to summarise the diversity and distribution of care traits across the squamate lineage, assign absence of SG based on the presence of other care behaviors (see above), as well as identify oviparous species displaying 'egg attendance', we organised similar forms of parental behavior into 5 broad categories that encapsulate the primary forms of care displayed by squamate reptiles (Table A4.4). These were nesting behaviour, egg manipulation, defense, neonatal assistance and post-partum parent-offspring associations.

For nesting behaviour, we only included reports where females directly and physically manipulated substrates into an egg-receiving site as nesting behavior. We did not include reports of mothers displaying selectivity for particular egg-laying site characteristics, as some degree of selectivity in site selection was considered to be ubiquitous across oviparous squamates. We defined a species as displaying parent-

offspring associations if offspring remained in social contact with a parent (typically the mother) beyond the time of hatching or parturition. To be assigned parent-offspring association, reports of overlap between adult and juvenile space use needed to be corroborated with observations of interactions and/or tolerance between adults and juveniles (e.g. juveniles in direct contact with an adult, adults and juveniles basking in close proximity, juveniles and adults found sharing crevice sites, burrows etc.). Oviparous species that displayed egg manipulation, defense or neonatal assistance where considered to display 'egg attendance' in all relevant analyses.

Table A4.4. Definitions of parental care behavior used to categories reports of care into different functional modes.

Care Mode	Definition
Nesting Behaviour	Construction of a nest space in which to receive eggs. This includes species that excavate nests and those that construct nests from leaf litter and debris. Does not include communal nesting.
Egg Manipulation	Any behavior involving parental contact or manipulation of the eggs. This includes brooding behavior, relocation of eggs during incubation and removal or consumption of non-viable eggs.
Defense	Any behavior in which parents are observed to respond defensively to potential predators approaching the eggs or nest.
Assist Neonates	Reports of parents assisting hatchlings out of their shell/birthing membrane during hatching/parturition.
Parent-Offspring Association	Sustained association or tolerance between parent(s) and offspring after hatching or birth. Includes active or passive defense of neonates, crevice or burrow sharing, and postponed dispersal in which offspring remain within the parental territory for an extended period.

Social grouping

The main aim of our review was to detect reports of social grouping between adults and juveniles (SG) indicative of tolerant social interactions among overlapping generations. Therefore we accepted reports of adults grouping together with 'juveniles', 'hatchlings', 'neonates', 'offspring' and 'young' but not 'sub-adults', or 'yearlings'. To confirm reports, we conducted individual searches on all species reported to show parent-offspring

association (Table A4.4) or other grouping behaviour between adults and juveniles, entering the species name (including recent taxonomic revisions) as the search term and critically reviewing any relevant literature. In some cases, particularly within the review of Greene et al. [3] of parental care in vipers, reports of SG for some species have been compiled from personal communications with field herpetologists rather than from discrete published sources. Although lacking primary literature, these reports represent detailed observations from trained herpetologists and in many cases have been confirmed by subsequent studies [e.g. 4-6] and were therefore retained

Parity mode

We collected all data on the parity mode of species from supplementary materials in Pyron et al. [7]. We did not differentiate between viviparity and oviviparity, and excluded from analyses all species that were reported as displaying both oviparity and viviparity (e.g. *Zootoca vivipara*).

Assigning absence of social grouping

Phylogenetic analyses of character state evolution inevitably suffer from incomplete data sets (see [8,9] for a discussion of the difficulties associated with missing data). The challenge of missing data also extends to the assignment of true negatives. For example, aside from general statements about the paucity of sociality across the Reptilia, the absence of SG in any given species is almost never explicitly stated, resulting in potential biases being introduced into phylogenetic reconstructions [10]. Various methods have been proposed for reducing the influence of such missing data on model estimates [reviewed in [11]. However, depending on the availability and distribution of data points across the phylogeny, different methods can produce highly divergent model estimates [11] and a cautious approach is advised for any phylogenetic analyses with incomplete data coverage [12]. Therefore, to avoid over-reaching the limits of current data, we chose to address incomplete data coverage and assignment of the absence of social grouping in two ways. First, we used strict criteria based on comprehensive literature searches to assign absence of SG. Second, we repeated each analysis on two separate datasets in which different methods of assigning zeros were applied (see below) and checked for qualitative consistency between model outputs.

Literature Searches

We used ISI Web of Science to search article titles, abstracts and keywords for all combinations of the search terms 'reptile', 'lizard', 'snake', 'amphisbaenia' and 'squamate', with the terms 'natal/offspring dispers*', 'birth*', 'birth site', 'territor*', 'kin', 'social system', 'social interaction', 'post hatching', 'post birth' and 'reproductive ecology', yielding 3194 unique documents. We read each publication title returned by these search terms and critically accessed all relevant articles. Within each relevant article we scanned the abstract and methods sections to determine field or laboratory methods and searched for the terms 'young', 'offspring', 'juvenile', 'neonate', 'social', 'aggregat*', 'care', 'birth', 'hatch', 'parental' within the text. We scanned all paragraphs containing these terms and if an article was suspected to contain relevant information based on these searches, we read it in full.

In some cases authors explicitly stated that juveniles did not associate with adults [e.g. 13-15], or that mothers abandoned eggs or neonates immediately after oviposition or parturition [e.g. [3,16]. However in the majority of cases the absence of parent-offspring or adult-juvenile associations was not explicitly stated or could not be inferred directly from the text. Therefore we assigned SG as being absent in a species if it met one of the following three criteria: 1) Behavioral forms of parental care other than Parent-Offspring Association had been reported (Table A4.4), based on aforementioned literature searches, with no mention of associations between adults and juveniles in any of the literature accessed; 2) the species was considered a 'model' organism, defined as having a citation count of ≥100 peer reviewed publications, making it highly unlikely that such behavior would not have been reported if present. To conduct these searches we used Scopus to search article titles, abstracts and keywords, using species names as the search term. We used Scopus for these searches instead of ISI Web of Science because the KeywordsPlus function of ISI Web of Science inflated estimates of the number of articles directly relating to the species in question; 3) studies of life history, reproductive ecology, spatial ecology, or habitat use were available in which researchers conducted observations and/or field collections during periods of hatching or parturition, collected observations of both adults and juveniles, but did not report any social association between these age classes. We further refined this latter criterion by only including studies that used manual methods of animal capture (i.e. we excluded

studies relying solely on passive trapping) to ensure researchers had considerable opportunities for observing associations between adults and juveniles.

Alternative data sets

To construct the first dataset (conservative), we followed the methods outlined above: All species for which reliable reports of SG were available and which were included in the phylogeny, we coded as '1' (n = 84) and species meeting criteria for absence were coded as '0' (n = 240). For the second dataset (relaxed), we relaxed the criteria for assigning '0's for SG. Specifically, in addition to the zeros assigned in the first dataset we coded a species as '0' for SG if it belonged to a taxonomic family for which no reports of SG were found for any representative species during our literature search (n = 886). We chose complete absence of reports of SG at the family level because this was the highest taxonomic classification possible within the order squamata and therefore the most conservative approach for assigning zeros based on absence of reports. Given the relative rarity of SG among squamates and strong phylogenetic signal of the trait (Table 6.1) we consider this to be a reasonable assumption.

Statistical approaches

We used three different methods to test the hypothesis that SG among squamate reptiles has evolved more readily from a background of viviparity. The first method, binary phylogenetic generalized linear mixed modeling (PGLMM) implemented in the R package 'ape', provides coefficient estimates and statistical tests of each predictor in the model while accounting for phylogenetic signal in the response variable. Fit without predictors, these models provide a test for phylogenetic signal in the response [17]. Thus, we began by fitting a model with no predictor variables to test for phylogenetic structure in the distribution of SG across the tree (Table 6.1: signal in response values). We then fit a model including parity mode and citation count as predictors to confirm the influence of parity mode on the occurrence of SG after accounting for phylogenetic structure and that our data were not biased by available literature on each species, respectively (Table 6.1). To test the hypothesis that egg attendance also facilitates SG among oviparous species we repeated this procedure, fitting a model with egg attendance as a predictor variable in analyses restricted to oviparous species, but found no significant association (Table 6.1).

We used parametric bootstrapping to evaluate uncertainty in the intercept (β0), main predictor (β1) and phylogenetic signal (s2) estimated from each PGLMM. This was achieved by an iterative simulation procedure. We first simulated the evolution of each predictor variable (parity and egg attendance) across the phylogeny, specifying the rate of character change as that estimated by a two-state Markov model of ancestral character estimation based on the real data. Using these simulated predictor values and the parameter estimates from our original PGLMM fit, we simulated response data (i.e. presence absence of social grouping) and re-fit the model with these simulated predictor and social grouping values. We repeated this procedure 1000 times for the conservative data sets and 250 times for the relaxed data sets (reduced number of simulations due to computational limitations) then checked each original parameter estimate against the distribution of estimates for that parameter returned from the simulations (Figure A4.3). This allowed us to evaluate bias in our original parameter estimates (Table 6.1) and confirm that 95% of the distribution of simulated estimates for both β1 and s2 did not cross zero (Figure A4.3; Table 6.1).

Second, we used Multi-state Speciation and Extinction (MuSSE) models [18,19], implemented in the R package 'diversitree'. MuSSE models provide estimates of transition rates between different character states while accounting for variation in speciation and extinction rates associated with each state [18]. Accounting for such variation is potentially important in our context, as viviparity has been associated with comparatively high rates of speciation within the squamates [7]. We combined our two binary characters (parity mode: oviparous, viviparous; and social grouping: present, absent) into four unique character states (Figure A4.4), and then estimated transition rates between each character state based on maximum likelihood reconstructions [see 18]. We began by fitting a model that allowed transition rates from a state of 'SG absent' to a state of 'SG present' to vary depending on parity mode. We then fit a constrained version of the model in which these transition rates were forced to be equal, and compared model fits using a likelihood ratio test. If the model allowing transition rates to vary is favored by AIC this test indicates that transitions to SG have occurred at different rates in oviparous and viviparous ancestors.

Third, we performed ancestral state reconstructions via stochastic character mapping in the R package 'phytools' [20]. We chose stochastic character mapping over marginal or joint likelihood approaches because this method allows for more robust

estimation of error associated with parameter estimates by incorporating MCMC sampling. As analyses were performed on a maximum likelihood tree [21] we were not able to incorporate phylogenetic uncertainty into reconstructions by sampling from a posterior distribution of trees. Therefore we used an empirical Bayesian approach, in which we fit a continuous-time reversible Markov model for the evolution of each of our two binary traits (parity mode and social grouping) to the tree, and then simulated 1000 stochastic character histories for each reconstruction using the model fit and tip states [see 22]. This allowed us to estimate transition rates between states as well as the number of independent origins of social grouping from the posterior distribution of parameter estimates from each map set. Finally, we used this approach to estimate the strength of correlated evolution between viviparity and social grouping by calculating the similarity of reconstructions between map sets [20]. We then repeated this procedure to investigate the evolutionary correlation between parity mode and stable social grouping, as well as between egg attendance and social grouping among oviparous squamates.

For all analyses we used a recent time-calibrated squamate phylogeny [21] pruned to include only those species for which trait data were available. Thus, sample sizes differed between analyses conducted on the conservative and relaxed data sets (Table 6.1). We included tuatara (*Sphenodon Puctatus*) as an out-group in all analyses. Due to controversy over whether reversals from viviparity to oviparity are biologically plausible [10,23-27] we fit MuSSE models both allowing and prohibiting transitions from viviparity to oviparity and report the output of all models (Table A4.1). Additionally, because multiple transitions in adaptive traits are unlikely to arise simultaneously [28], simultaneous double transitions (i.e. from a state of oviparity without SG to a state of viviparity with SG) were also prohibited (Figure A4.4).

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Appendix V

Adaptive Responses to Cool Climate Promotes Persistence of a Non-Native Lizard

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Abstract

Successful establishment and range expansion of non-native species often require rapid accommodation of novel environments. Here, we use common garden experiments to demonstrate parallel adaptive evolutionary response to a cool climate in populations of wall lizards (*Podarcis muralis*) introduced from southern Europe into England. Low soil temperatures in the introduced range delay hatching, which generates directional selection for a shorter incubation period. Non-native lizards from two separate lineages have responded to this selection by retaining their embryos for longer before oviposition - hence reducing the time needed to complete embryogenesis in the nest - and by an increased developmental rate at low temperatures. This divergence mirrors local adaptation across latitudes and altitudes within widely distributed species and suggests that evolutionary responses to climate can be very rapid. When extrapolated to soil temperatures encountered in nests within the introduced range, embryo retention and faster developmental rate result in one to several weeks earlier emergence compared with the ancestral state. We show that this difference translates into substantial survival benefits for offspring. This should promote short- and long-term persistence of non-native populations, and ultimately enable expansion into areas that would be unattainable with incubation duration representative of the native range.

Introduction

Non-native populations often encounter novel environments that impose strong directional selection. They therefore provide useful systems to study the process of adaptation [1-6]. However, evidence for adaptive divergence between native and non-native populations is currently limited, in particular for vertebrates [1]. Furthermore, the selective agents responsible are typically inferred from the pattern of phenotypic divergence, rather than being independently demonstrated, which makes it difficult to rule out non-adaptive processes [7]. The most convincing cases of adaptation in non-native species are therefore those where the causes of selection can be identified and the responses mirror locally adapted phenotypes within native ranges.

Recent studies of introduced insects and plants suggest that adaptive responses can facilitate spread into environments that were previously too stressful [8-10]. As climatic similarity is often the best predictor of establishment success of non-native species [11-13], climate is likely to exercise strong selection on many non-native populations. Such populations may therefore remain small and geographically isolated until they evolve tolerance to the new climatic regime, following which rapid range expansion is made possible. Understanding the mechanisms by which populations respond and adapt to novel climatic conditions is therefore crucial for predicting both the persistence and expansion of non-native species.

The common wall lizard, *Podarcis muralis,* is native to southern and western Europe but has been introduced multiple times to Germany, England, and North America over the past 100 years [14-16]. These introduction sites represent different climatic conditions compared to the native range [14]. For example, air temperatures during the main activity season in populations in England are 5 - 10°C lower than their source regions in Tuscany and Western France (Supplementary Fig. 1). Although behavioural thermoregulation enables non-native lizards to maintain annual activity patterns similar to those in their native range, these climatic differences put soil temperatures well below what females prefer for their nests (Supplementary Table 1). Such cool conditions retard the rate of embryonic development [17], resulting in delayed hatching and even failure to complete embryogenesis before winter in cool summers [18]. Thus, we should predict strong selection for shorter incubation duration in non-native wall lizard populations, in particular at the cool temperatures rarely

encountered in the native range but that are frequent in introduced populations in England.

Incubation duration can be reduced through several different mechanisms. Firstly, embryos may be more advanced at oviposition. By capitalizing on the female's ability to maintain body temperatures substantially higher than those of nests this reduces the overall time for completion of embryogenesis [19]. Egg retention is evolutionarily labile in lizards and has been shown to exhibit counter-gradient variation within and between species [20-24]. Secondly, embryos can evolve a faster development rate. For example, in *Sceloporus undulatus*, embryos from populations in cooler climates have a higher cardiac output and hence a more efficient delivery of nutrients and oxygen that promotes faster embryogenesis [25]. Finally, embryos may hatch at an earlier developmental stage or at a smaller size, and capitalize on residual yolk for completion of growth after emergence [26, 27].

Here we designed a set of common garden experiments to test the hypothesis that non-native populations have adapted to the cool climate in their non-native range. First, we show that soil temperatures in the non-native range are physiologically stressful and impose significant selection on shorter incubation duration. Second, we test if non-native populations have responded to this selection and establish the underlying mechanisms of adaptation. Finally, we use developmental reaction norms to infer the implications of shifts in incubation duration for the timing of hatching and recruitment under naturally fluctuating soil temperatures in the non-native range. Our results demonstrate prolonged embryo retention and faster embryonic growth at low temperatures in introduced wall lizard populations and suggest that these responses will have significant implications for the long term persistence of this species in England.

Materials and Methods

The common wall lizard is a small (approximately 50-70mm snout to vent length) diurnal lizard. It is native to southern and western Europe, but has established non-native populations in many places in Europe and in North America [14, 15, 28]. There are around 30 extant populations in southern England, the majority of which are highly geographically restricted with limited ecological impact [15].

(a) Female thermal preference and soil temperature

We first established preference for nest sites under unrestricted thermal conditions and in field enclosures. Unrestricted conditions were established by creating a thermal gradient in a large cage (1070 × 480 × 420 mm) of approximately 45 °C to 20 °C during the peak in the afternoon, falling to 15 °C at night (Table S1). Five females were used in this experiment. Females were inspected in the morning and in the afternoon for signs of egg laying. Once they had laid, we recovered their clutch and replaced it with a thermal logger (thermochron Ibutton, model number DS1921G) that logged the temperature for 5 consecutive days. A second data set was generated from females housed in outdoor enclosures where suitable nesting sites with naturally variable thermal properties were available. Five female lizards were placed into the enclosure and allowed to lay eggs. The temperatures of nests were monitored using thermal loggers for 35 days following oviposition. The temperatures of these nest sites were compared to possible nest sites across the enclosure. Further details on these experiments are provided in the Supplementary Information.

(b) Effects of hatching date on recruitment into adulthood

In 2012, we released 288 hatchlings from females of both French and Italian origin in a disused quarry close to the original non-native populations on the Dorset coast (50.59°N, -2.01°E). All eggs were incubated at constant 24 °C. This temperature is at the high end of the average expected temperature of nests in England but at the low end of temperatures in the native range (Supplementary Table 1; [29, 30]). Upon hatching, offspring were weighed to the nearest 0.01g and measured snout to vent length and total length to the nearest 0.5mm with a ruler. They were toe clipped for individual identification. Individuals were released in four batches, the timing of which represented a compromise between limiting the number of days offspring spent in captivity while maximizing the number of hatchlings released at a single field trip. The releases occurred on July 15 (offspring hatching from July 9 until 15), July 24 (July 16 – July 24), August 6 (July 26 – Aug 6) and August 21 (Aug 6 until Aug 14).

Lizards were recaptured in their second year (i.e., first year as adults) by repeated visits during the breeding season from late March until late June (n = 10). Upon recapture individuals were measured for SVL and mass (as above) before being temporarily marked with a marker pen before release to avoid unnecessary recapture.

Overall, we recorded 41 individuals from the 288 released hatchlings, representing a recapture rate of 14% over the first two years of life. We used the recapture data to test how the timing of hatching affects recruitment into adulthood using models with a categorical variable with three levels representing release batch, lineage (Italian vs French), and their interaction as fixed effects and included days in the laboratory and mass at hatching as covariates. Because the last release included only 27 offspring from seven clutches, we pooled the last two release batches to avoid having one level represented by very limited data. Furthermore, because our sample size is modest, we could not meaningfully take family effects into account (9 of 65 families produced 2 recruits, the rest one or none). We therefore treated all offspring as independent for this analysis.

(c) Establishing differences in incubation duration between native and introduced populations

(i) Experiment at constant temperatures (20 versus 24 °C):

We caught one hundred and twenty two lizards by noosing at the early stages of gestation in early spring 2013 at three locations in Tuscany (Greve in Chianti (43.58°N, 11, 31°E), Prato (43.91°N, 11.10°E), Colle di Val d'Elsa (43.42°N, 11.11°E)), four in Western France (Dinan (48.45°N, -2.07°E), Josselin (47.95°N, -2.54°E), Pontchateau (47.43°N, -2.09°E), Pouzagues (46.78°N, -0.44°E)) and four in southern UK (Italian origin: Ventnor Town (50.59°N, -1.21°E), Ventnor Botanical Garden (50.58°N, -1.22°E); French origin: Cheyne Weare (50.53°N, -2.43°E) and East Portland (50.54°N, 2.42°E)). The non-native populations represent at least two separate native sources for both the Italian and the French lineage that correspond well to the sampling locations of native animals [15]. Females had visible mating scars, large follicles or carried recently ovulated eggs (as determined from palpation), which ensured that they were fertile but would complete the large majority of gestation in the laboratory.

Females were housed individually in cages (590×390×415 mm) with sand as substrate, bricks as shelter and a water bowl, and held at a light cycle of 12:12 hours of daylight:darkness. They were given access to basking lights (60W) for 8 hours per day and a UV light (EXO-TERRA™ 10.0 UVB fluorescent tube) for 4 hours per day and they were fed mealworms and crickets daily. Cages were inspected in the morning and in the late afternoon for signs of oviposition (which is easily assessed by visual inspection of

females), such that eggs were recovered within 12 hours of oviposition. Once laid, clutch size was assessed and the clutch and female were weighed to the nearest 0.01g. All data in this paper are from the first clutch produced by females in that year (females can lay up to three clutches per year). The median lay date for native females was two weeks earlier than introduced females of both lineages (introduced French females: 30th May, native French females: 13th May, introduced Italian females: 29h May, native Italian females: 13th of May).

For 65 clutches (each with more than four eggs) we selected one egg for dissection to assign the embryo to a developmental stage according to the scheme for Lacertids [31]. For embryos that exhibited characters of two consecutive stages we assigned an intermediate stage (e.g., 26.5). All staging was conducted by a single person (JW) who was unfamiliar with the coding system and hence embryos were scored blindly with respect to origin (native *vs.* introduced) and lineage (French *vs.* Italian). The remaining eggs were split into two groups and put into small plastic containers filled two-thirds with moist vermiculite (5:1 vermiculite:water volume ratio; changed every 20th day) and sealed with clingfilm. Half the clutch was incubated at constant 20 °C and the other at constant 24 °C in standard refrigerated incubators fitted with water baths to maintain humidity. During incubation we measured the heart rate of all embryos within each of the 122 clutches as an estimate of cardiac output and hence developmental rate [32]. Following previous work [25, 32], heart rate was measured using a digital egg monitor (Buddy, Avitronics, England) in a constant temperature room set to either 20 or 24 °C (matching the incubation temperature of eggs). This was done twice during incubation, at 35 and 65 days following oviposition in the 24 °C treatment and at 20 and 40 days in the 20°C treatment.

Eggs were inspected daily for signs of hatching (i.e., pipping) and the hatching date refers to the day of emergence. At hatching the offspring were measured snout-to-vent length and total length to the nearest mm with a ruler and mass was recorded to the nearest 0.01g.

Because of the limited sample size per population (between 5 and 17 clutches) we pooled populations into the four main categories (Native Italian, Native French, Non-native Italian, and Non-native French). We analysed differences in clutch size and the embryonic stage at oviposition between lineages (French *vs.* Italian) and origin (Native *vs.* Non-native). Because of the very large difference in incubation duration and heart

rate in the 20 and 24 °C treatment (122.80 ± 0.60 days *vs.* 56.15 ± 0.28 days, 47.45 ± 0.34 beats/minute *vs.* 76.23 ± 0.51 beats/minute) we analysed these variables separately for the two temperature treatments (heart rate was averaged across the two measurements for each clutch and temperature [25, 32]). In contrast, hatchling size variables overlapped between incubation treatment and we therefore fitted a single model with lineage, origin, and temperature to the mean hatching mass per clutch and treatment, including female identity as a random effect. All linear models were fitted in R using the car package to generate Type III F-tests of fixed effects. When the interaction(s) was non-significant, results for main effects are presented from models excluding the interaction.

(ii) Experiment at shifting temperatures:

To assess differences in incubation duration at temperatures too low for completion of embryogenesis, we designed a second experiment shifting eggs between temperatures. For this experiment, we only used animals of Italian origin, collecting gravid females in spring 2014 at three locations in Tuscany (Greve in Chianti (43.58°N, 11, 31°E), Peccioli (43.55°N, 10.72°E), Colle di Val'Elsa (43.42°N, 11.11°E)) and the same two focal non-native Italian populations as in the above experiment (Ventnor Town and Ventnor Botanical Garden). All animals were handled and treated as above. Clutches were split into four categories; (1) constant 28 °C (n = 50), (2) 15 °C for 14 days followed by constant 28 °C (n = 50), (3) 18 °C for 14 days followed by constant 28 °C (n = 45), and (4) 28 °C for 14 days, followed by 18 °C for 14 days, and finally 28 °C until hatching (n = 40). The last treatment was included to address if there were any marked differences for embryos exposed to cool temperature in early versus mid-development [33]. Eggs were inspected for hatching around the same time daily (in the late afternoon) and hatchlings were measured as described above.

We calculated for each clutch the differences in incubation duration between eggs at constant 28 °C and eggs at the other treatments, and used these estimates as our response variable. A significant difference between native and non-native populations would be interpreted as faster (or slower) developmental rate at cool temperatures. Such effects may be more or less pronounced if embryos adjust their developmental rate to conditions experienced early in gestation [19, 34]. Eggs incubated at 18 °C for 14 days before being transferred to 28 °C took on average half a day longer to hatch than

eggs from the same clutch that were exposed to the 14 days 18 °C treatment in mid-gestation (46.5 vs 45.9 days; paired t-test, t = 2.99, p = 0.01, df = 28). However, because we were primarily interested in the overall effect between native and non-native populations we use the average of the two treatments in our analyses and for fitting thermal reaction norms (see below), which maximizes sample sizes when embryos in one of the 18 °C treatments failed to hatch.

To test for differences in preferred body temperature between native and introduced gravid females, in 2014 we recorded the body temperature of 72, individually housed, captive females from both native Italian and non-native UK populations (same sources as described above) eight times a day over a three-day period using an infrared thermometer. This has been demonstrated to be a reliable measure of internal body temperature in small lizards [35]. Measurements began one hour after basking lights came on and continued on the hour until basking lights turned off. At night temperatures dropped to 15°C across all cages. Thus, there was limited potential for females to alter gestational temperature via shelter site choice. We analysed differences between native and introduced females using linear mixed models with body temperature as the response variable, origin (native or introduced) as the predictor variable and individual ID and observation day as random effects.

(d) Predicted hatching success in the UK

Temperature-dependent developmental rate at constant temperatures can be used to predict incubation duration at fluctuating temperatures [36-38]. To predict incubation duration of our non-native wall lizard populations we used our data on developmental rate at constant temperatures at 20, 24 and 28 °C and the estimated developmental rate from the experiment switching eggs between 15 or 18 and 28 °C (all for the Italian lineage; see SI for further details). Data on developmental rate at higher temperatures were collected from the literature assuming that the developmental rate approaches a species-specific maximum at 35 °C, data for which was provided by incubation experiments on a population from northern Spain (belonging to the same lineage as our French populations; [39, 40]). Given that the difference between lineages and origins is small already at 24 °C and that high temperatures are rarely encountered in the UK (see above) this should not bias our estimates. We fitted a four-parameter Weibull function and verified that it performed well for predicting incubation duration using

experimental incubation under a daily fluctuating temperature regime similar to that of natural nests in England (see SI for details).

We used soil data for locations south of the 53rd parallel north obtained from the British Meterological Office Integrated Data Archive System, Land and Marine Surface Stations Data for the period 2002-2013. We chose this period because of the availability of recent and yearly data across a range of consistent locations that are representative of annual variation, including relatively warm (e.g., 2006) and cool (e.g., 2011) summers, and that covers the range of known wall lizard introductions in the UK. For exach of the sites, soil temperatures are recorded every hour. We used the period from May 15 to September 15 in our analysis as the first is representative of the timing of oviposition for non-native lizards in the wild (the median lay date for females captured in the middle or end of gestation and brought to our laboratory for oviposition is the 16th of May; Supplementary Table 2) and hatching past mid-September is unlikely because of the rapid decrease in daily soil temperature maximum (soil temperatures exceed 20 °C – the minimum constant temperature for successful hatching in the lab – less than 0.3% of the time following the 15th of September across all years and sites in southern England). In addition, this four month period is approximately equal to the predicted incubation duration for sites close to extant non-native populations and is in line with observations of newly hatched offspring in populations in England and at the northern native range limit ([41], T. Uller & G.M. While, pers obs; Tony Pashley, pers. comm.).

To generate expected hatching dates we calculated the average proportion of time per day spent at different temperatures over this period and used this and the developmental rate data to generate predicted incubation durations for each location and year (see SI for details).

Results

(a) Female thermal preference and soil temperature

Typical nesting sites in the introduced range are substantially cooler than that preferred by females for their nests under unrestricted thermal conditions (Supplementary Table 1). We fitted thermal reaction norms for developmental rate of native Italian lizards to soil temperatures at depths representative of nesting sites in southern England (obtained from the UK Meteorological Office). This predicted incubation times well over three months even in relatively warm years, and failure to complete embryogenesis before winter in cooler years (Supplementary Table A5.1; Supplementary Fig. 2).

Table A5.1: Recruitment of offspring into the breeding population as a function of offspring hatching date. Offspring were released in four batches corresponding to offspring hatch date. Because the last release included only 27 offspring from seven clutches, we pooled the last two release batches.

Release batch	Hatch dates	Proportion of offspring recruited
1	9th July - 15th July	16.50%
2	16th July - 24th July	20.50%
3	25th July - 21st August	7.60%

(b) Effects of hatching date on recruitment into adulthood

There were strong effects of hatching date on juvenile recruitment into adulthood. Even under benign incubation temperatures that result in hatching in mid- to late summer, a two to three week difference in hatching translated into substantially reduced recruitment into the breeding population (logistic linear model: $\chi^2 = 7.4$, $P = 0.02$; Table A5.1). Combined these results suggest strong selection for shorter incubation duration in non-native wall lizard populations.

(c) Divergence in incubation duration between native and introduced populations

There was no difference in female snout-to-vent length between origins (native *vs.* non-native; $F_{1,118} = 0.01$, P = 0.92) or lineages (French *vs.* Italian; $F_{1,118} = 1.63$, P = 0.20). Females from non-native populations produced larger clutches than females from native populations and Italian females produced larger clutches than French females (origin: $F_{1,117} = 8.73$, P = 0.004; lineage: $F_{1,117} = 12.96$, P < 0.001; SVL: $F_{1,117} = 63.4$, P < 0.001). Eggs from French females were heavier than eggs from Italian females and tended to be smaller in non-native populations of both origins (origin: $F_{1,118} = 5.37$, P = 0.022; lineage: $F_{1,118} = 3.31$, P = 0.072). A total of 521 eggs were produced of which 468 hatched. Embryonic mortality did not differ between origins ($\chi^2 = 1.02$, P = 0.31) or lineages ($\chi^2 = 0.19$, P = 0.66).

Incubation duration was strongly affected by incubation temperature, and it was significantly shorter in non-native populations of both Italian and French lineages at 20 °C but not at 24 °C (Table A5.2; Fig. A5.1). Embryos of the Italian lineage hatched sooner than embryos of the French lineage at both temperatures (Table A5.2; Fig. A5.1). Egg mass did not affect incubation duration at 20 °C, but larger eggs had shorter incubation duration at 24 °C (Table A5.2). The shifting incubation temperature experiment confirmed shorter incubation duration for non-native animals at both 15 and 18 °C. Eggs from non-native females of Italian origin exhibited a 12.7 ± 0.33 day delay in incubation duration when held at 15 °C for 14 days (compared to eggs held at a constant 28 °C days) compared to a delay of 13.6 ± 0.14 days for eggs from native females ($F_{1,36} = 7.91$, P = 0.007). The same pattern was observed at 18 °C (non-native populations = 10.9 ± 0.21 day delay; native populations = 11.5 ± 0.18 day delay; $F_{1,36} = 4.14$, P = 0.049).

Embryos from non-native populations were significantly more advanced at oviposition compared to embryos from native populations, and this pattern was consistent for both lineages ($F_{1,55} = 11.10$, P = 0.002, Fig. A5.2). Differences in embryonic stage are unlikely to be explained by exposure to higher temperature before oviposition as there was no difference in gestational body temperature of non-native and native females (tested in the Italian lineage only: $\chi^2 = 0.92$, P = 0.34). Heart rate showed a more complex pattern, with a predicted higher heart rate in non-native populations of the French lineage compared to their native populations, but no

difference in embryos from the Italian lineage. This lineage by origin interaction was significant at 20 °C ($F_{1,86}$ = 7.64, P = 0.007; Fig. 3) but failed to reach statistical significance in the 24 °C treatment ($F_{1,85}$ = 3.71, P = 0.051; Fig. 3). Refitting models of incubation duration with embryonic stage at oviposition as an additional predictor confirmed that it significantly contributed to the shorter developmental time at both 20 °C ($F_{1,43}$ = 13.72, P < 0.001) and 24 °C ($F_{1,47}$ = 16.59, P < 0.001). This was not the case for heart rate, which failed to significantly predict incubation duration at either temperature in refitted models ($F_{1,81}$ = 1,38, P = 0.24 and $F_{1,84}$ = 1.89, P = 0.17 respectively). In both cases the difference between non-native and native populations at 20 °C remained statistically significant (P <0.001). Finally, Italian offspring were smaller than French offspring ($F_{1,86.4}$ = 10.28, P = 0.002) and both lineages had smaller hatchlings at 20 °C compared to 24 °C ($F_{1,52.9}$ = 113.41, P < 0.001), but offspring from non-native populations hatched at a similar size to offspring from native populations ($F_{1,85.7}$ = 1.41, P = 0.24).

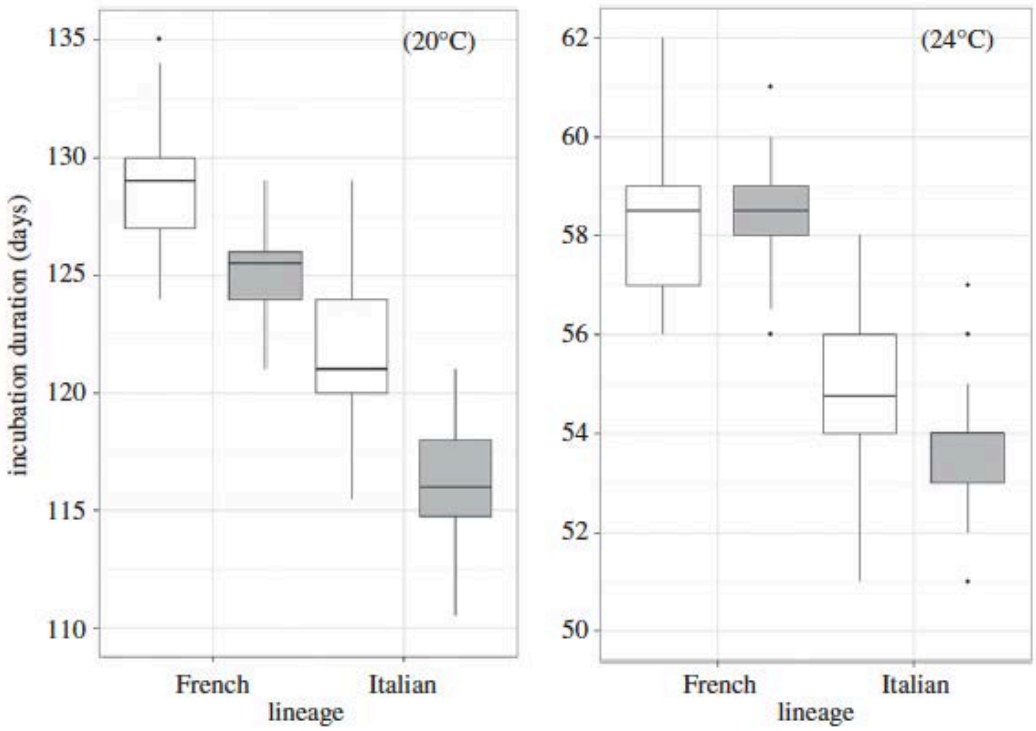

Figure A5.1. Incubation duration of eggs kept at constant 208C or 248C from female wall lizards of French and Italian lineages sampled from native (white fill) and non-native (grey fill) populations.

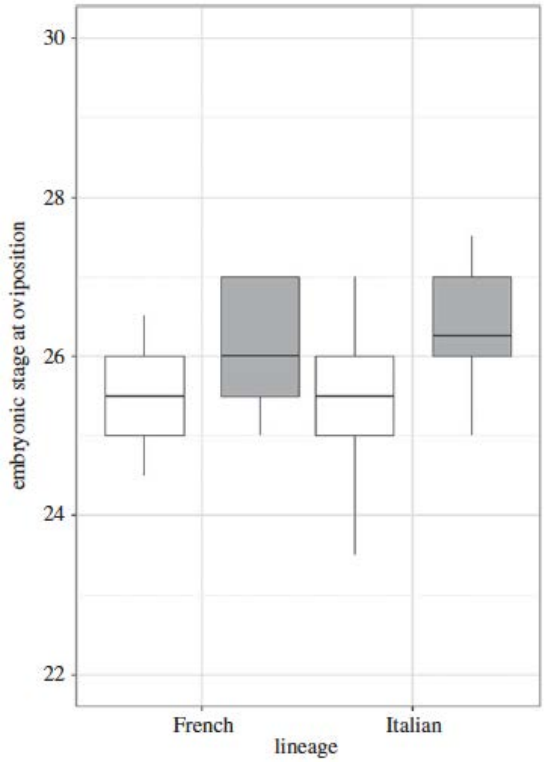

Figure A5.2. The embryonic stage of development at oviposition for eggs laid by French and Italian female wall lizards from both native (white fill) and non-native (grey fill) populations.

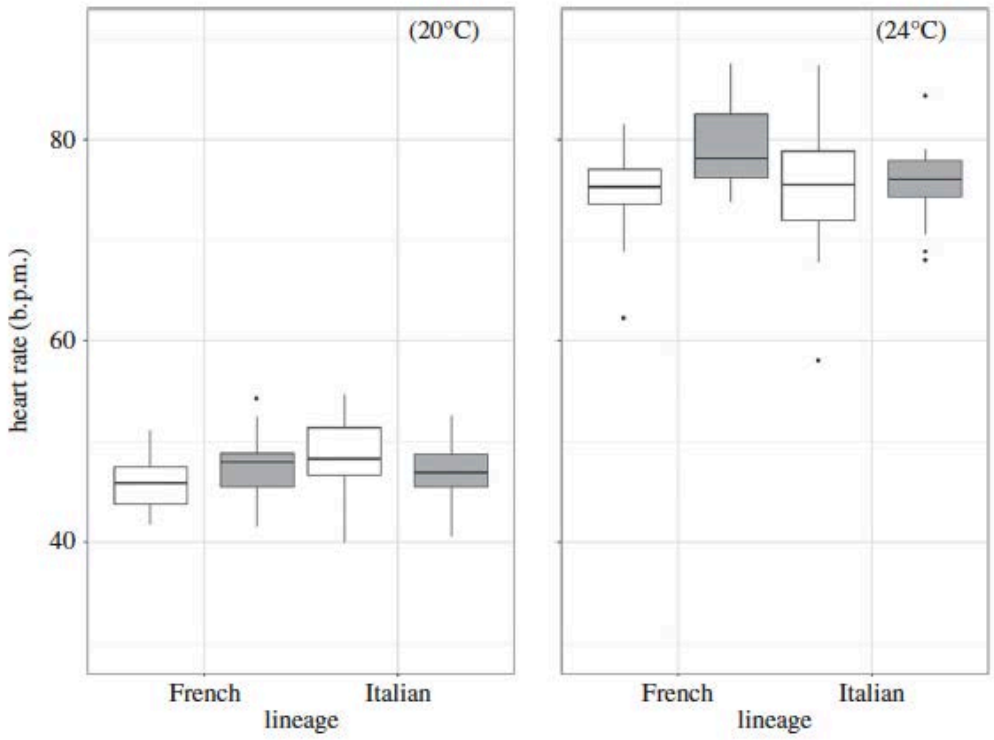

Figure A5.3. The heart rate (b.p.m.) of developing embryos of eggs kept at a constant 208C or 248C from female wall lizards of French and Italian lineages sampled from native (white fill) and non-native (grey fill) populations.

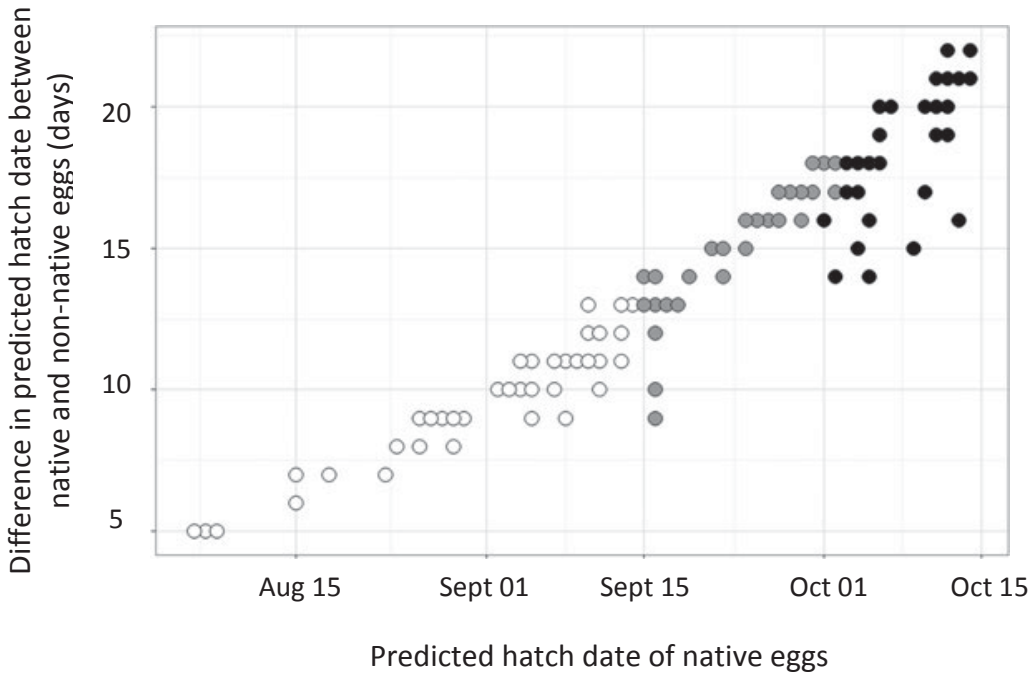

Figure A5.4. The predicted difference in the timing of hatching of eggs from native versus non-native wall lizard populations of the Italian lineage as a function of the estimated timing of hatching for native developmental rates. Estimates are based on soil temperatures across 34 sites in southern England over a 10-year period and a laying date of 15 May (data truncated at 15 Oct). Assuming hatching is unlikely past 15 Sept (see the electronic supplementary material), white dots represent soil temperatures that would allow successful hatching for both native and introduced lizards, grey dots represent soil temperatures that would allow successful hatching only for lizards from introduced populations and black dots represent soil temperatures that would not allow hatching for either native or introduced lizards.

Table A5.2. Output from linear models examining the effect of lineage and origin on the incubation duration of eggs kept at 20°C or 24°C. Statistically significant p-values are in bold. Main effects reported from model excluding interaction.

	20°C			**24°C**		
Factor	**d.f.**	***F***	***P***	**d.f.**	***F***	***P***
lineage	1,96	151.77	**<0.001**	1,99	197,23	**<0.001**
origin	1,96	47.41	**<0.001**	1,99	3.07	0.08
egg mass	1,96	0.83	0.36	1,99	4.27	0.04
lineage x origin	1,95	1.68	0.19	1,98	0.74	0.39

(d) Consequences for the timing of hatching

To estimate the consequences of these responses for the timing of hatching we modelled the predicted incubation duration of non-native and native lizards (of Italian origin) based on naturally fluctuating soil temperatures in sites representative of nesting locations across 34 sites in Southern England. Predicted hatch dates for non-native eggs were one to three weeks earlier compared to the ancestral state, which greatly increased the chances of completing embryogenesis before autumn (Fig.4).

Discussion

Our common garden experiments demonstrate parallel reduction in incubation duration of embryos in wall lizard populations following independent introduction to cooler climate. This is consistent with adaptive evolutionary responses to the relatively cool nest temperatures in the introduced range, which necessitates sustained development at temperatures well below 24 °C to complete embryogenesis (only 3% of soil temperature recordings at soil depths representative of nests in southern England are above 24 °C for the relevant incubation period, i.e., from 15th of May to 15th of September). While embryo retention and faster developmental rate have evolved repeatedly in lizards in cool climates [21-24], our results demonstrate that such adaptations can arise very rapidly. Extant wall lizard populations in England were introduced less than 100 years ago, with the target populations tracing their origins back to between two and eight decades [15]. Thus, the results are consistent with recent evidence that geographic clines in introduced insects and plants can evolve within tens of generations (e.g., [42, 43]), and with studies of vertebrates that have demonstrated adaptive divergence across a similar number of generations [2, 3, 5, 44-47].

The reduction in incubation duration in non-native wall lizards appears to have multiple causes. Embryos in non-native populations are more advanced at the time of egg laying, which reduces the overall time to complete embryogenesis in the nest. We can rule out facultative egg retention as all females were housed under identical conditions and there was no difference in selected body temperature between native and introduced females (in addition, previous work has failed to experimentally demonstrate plasticity in egg retention in *P. muralis* [30], but see [24]for evidence from

the skink *Bassiana duperreyi*). However, egg retention cannot fully account for the reduction in incubation duration in non-native populations, nor does it account for the difference between lineages, as both lineage and origin explained significant amounts of variation even when the differences in embryo stage were controlled for statistically. Embryos from non-native populations must therefore also develop faster at and below 20 °C. Interestingly, there was no, or limited, increase in development rate at 24 and 28 °C. These responses thus demonstrate adaptive evolution of the slope and curvature of thermal reaction norms, which appears to be common for population divergence in plasticity [48].

What are the mechanisms underlying faster developmental rate in non-native populations? Eggs were somewhat smaller in non-native populations, but egg size cannot explain differences in incubation time between non-native and native populations (in fact, larger eggs hatched earlier at 24 °C). Other maternal effects on yolk composition could be involved and the relative contribution to changes in yolk nutrients versus intrinsically up-regulated metabolism in embryos warrants further study. The increased heart rate of embryos from non-native populations of the French lineage suggests that a faster development may partly be due to increased cardiac output [32]. This mechanism has been shown to account for faster developmental rate at high latitudes in *Sceloporus* lizards [25]. However, heart rate itself was a poor predictor of incubation duration across our populations and lineages, suggesting that the divergence between non-native and native populations in the rate of development is not well accounted for by such simple estimates of nutrient and O_2 delivery to developing tissues. The same applies to differences between lineages. Data from Spanish populations close to the presumed ice age refugia for the French lineage have even slower developmental rates at cool temperatures than our native French populations, [39], suggesting there may be a phylogenetic signal to developmental rate, which persists in non-native populations. .

The shorter incubation duration in non-native populations should facilitate their long term persistence and increase their ability for range expansion. By predicting incubation duration of non-native and native lizards from soil temperatures across the introduced range we show that the combined effect of egg retention and faster embryonic growth should lead to one to three week earlier emergence compared to the ancestral state. Even two weeks earlier hatching, which is a common prediction from

the data, can make the difference between successful hatching and failing to hatch before the onset of autumn. Our recapture data show this also constitutes a substantial (e.g., two-fold) increase in survival after emergence (see also [30]). This increased survival of early hatched offspring could be the result of several non-mutually exclusive mechanisms, including increased opportunity for growth and production of fat bodies prior to hibernation ([49] see also [50]), positive effects of high embryonic temperature on physiological and morphological traits [51, 52] or greater ability to capitalize on seasonally available food sources [53]. Earlier emergence and long-term persistence of non-native populations could be further enhanced if non-native lizards also initiated reproduction earlier than their native counterparts. However, the extent to which there may have been corresponding responses in female characters that promote egg laying early in spring in non-native populations is currently unknown.

The shorter incubation duration should increase short- and long-term persistence of non-native populations and may enable expansion into areas that would be unattainable with incubation durations representative of the native range. A time delay between introduction and range expansion appears to be a common pattern in biological invasions and recent studies of non-native insects and plants have demonstrated how adaptive divergence can facilitate spread into environments that were previously too stressful [8, 9]. Wall lizards in England show limited natural dispersal [15] but the ability to recruit from nests with less benign thermal profiles may contribute to their expansion in several locations. Non-native populations with shorter incubation duration may also serve as sources for new introductions and hence make human-mediated range expansion more likely [54].

In summary, prolonged embryo retention and faster embryonic growth at low temperatures in non-native wall lizards suggest rapid adaptation following introduction to cool climate. We show that these responses have significant effects on recruitment and hence are likely to contribute to the survival and eventual range expansion of the species in its introduced range.

Data accessibility

All data associated with this MS will be made available on dryad.

Competing interests

We have no competing interests.

Author contributions

TU and GMW conceived of and managed the project, collected and analysed data, and wrote the paper. JW, GP, TH, BF, BH, and SM collected data for different parts of the project, and NJB assisted with analysis of climatic data. All authors commented on the paper.

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Appendix VI

An Experimental Test of Relatedness-Based Mate Discrimination in a Social Lizard

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Abstract

One of the major decisions individuals of many species make when deciding who to mate with is whether or not to inbreed. The prevailing theory is that individuals should avoid mating with closely related individuals because of the fitness costs associated with decreased offspring quality. However, theoretical work has suggested that levels of inbreeding depression need to be considerable for these costs to outweigh the possible, kin selected, benefits of inbreeding. While evidence for a preference for inbreeding exists in the literature, empirical results currently lag well behind theory. Here we conducted an experiment to examine mate discrimination and preference with respect to relatedness in a natural population of the family living lizard, *Liopholis whitii*. We show that both male and female *L. whitii* distinguish between related and unrelated individuals and, furthermore, that both sexes preferentially associate with more closely related members of the opposite sex. These results correspond closely with patterns of pairing observed in the wild where both males and females have been shown to choose mating pairs that are more closely related to them than expected by chance. Combined, our findings suggest that mate choice in this system may represent an active preference for mating with related individuals rather than a result of passive constraints associated with population viscosity.

Introduction

Mate choice is a fundamental process which has the potential to drive the evolutionary and ecological trajectory of populations (Keller and Waller 2002; Charlesworth 2006). One of the major decisions individuals of many species face when deciding who to mate with is whether or not to inbreed. Because inbreeding has the potential to have a number of negative consequences for offspring fitness (e.g. through the presence of deleterious homozygous alleles or the loss of heterosis; Keller and Waller 2002; Charlesworth and Willis 2009) the prevailing theory has been that individuals should avoid mating with closely related individuals. In line with this, substantial empirical work across a range of taxa has shown that individuals often have a preference for unrelated partners (e.g. Bretman et al. 2009; Fitzpatrick and Evans 2014; Fitzpatrick et al. 2014; Liu et al. 2014). Where preferences for inbreeding have been observed, they tend to be attributed to constraints on mating opportunities or an inability to recognize kin rather than an active preference for related partners (Pusey and Wolf 1996; Keller and Arcese 1998; Oh 2011).

This focus on inbreeding avoidance ignores a number of key points. First, there are costs associated with avoidance of inbreeding such as those related to dispersal or missed opportunities for mating (Keller and Arcese 1998; Kokko and Ots 2006; Olson et al. 2012). Second, inbreeding and inbreeding depression are expressed in different generations. Therefore, selection against being inbred (as affected by inbreeding depression) does not necessarily correspond with selection for inbreeding avoidance (which also depends on the lifetime numbers of inbred and outbred offspring produced) (Reid et al. 2015). Finally, inbreeding may actually be beneficial, as it provides closely related individuals with additional breeding opportunities and because parents are more closely related to inbred offspring than they are to outbred offspring. Thus, in many cases individuals stand to gain substantial inclusive fitness benefits by choosing to mate with more closely related partners (Parker 1979; Smith 1979; Bateson 1983; Waser et al. 1986; Puurtinen 2011; Duthie and Reid 2015; Reid et al. 2015). Indeed, theoretical work suggests that levels of inbreeding depression need to be considerable for its costs to outweigh its possible, kin selected, benefits (Kokko and Ots 2006), thus inbreeding preference should be the rule rather than the exception. However, while evidence for a preference for inbreeding exists in the literature (e.g. Kleven et al. 2005;

Thünken et al. 2007; Langen et al. 2011; Wang and Lu 2011; Robinson et al. 2012), empirical results currently lag well behind the theory.

Here we examined the extent to which mate discrimination is influenced by genetic relatedness in a family living lizard, *Liopholis whitii*. *L. whitii* live in stable family groups consisting of a long-term socially monogamous male-female pair and their offspring, who delay dispersal and remain within their parents burrow system for a year sometimes two (Chapple and Keogh 2005; While et al. 2009). Previous research has shown that that social pairs are significantly more related to one another compared to a random pairing of individuals in the population (While et al. 2014). Furthermore, when undertaking extra-pair copulations (which account for 30% of offspring; While et al. 2009a, b, 2011), males and females breed with individuals that are significantly less related to themselves than their social partners (While et al. 2014). These results suggest that social partner choice in this system may be constrained by strong genetic structure at a local scale and that extra-pair mate choice acts as a mechanism to alleviate this constraint (While et al. 2014). However, there are several factors that suggest caution with this interpretation. First, levels of inbreeding depression at the population level are extremely low, and are not significantly different from zero, suggesting limited costs associated with inbreeding (While et al. 2014). Second, pair relatedness does not predict whether individuals undertake extra-pair copulations, suggesting that extra-pair paternity might not be a mechanism to compensate for pairing with a closely related individual (see also Wang and Lu 2011). Finally, although extra-pair offspring are significantly more heterozygous than within-pair offspring, there is no evidence that this heterozygosity is correlated with estimates of fitness (While et al. 2014). Additional work is therefore required to distinguish the extent to which pairing with closely related individuals is a result of passive constraints associated with population viscosity or is an active preference for mating with related individuals.

To address this we conducted an experiment during the mating season that allowed us to examine mate discrimination and preference with respect to relatedness for both males and females and to compare this to the patterns of pairing observed from our natural population of *L. whitii* (see While et al. 2014). We used a Y-maze choice design to determine a) if individuals discriminate between related and unrelated individuals (measured via rate of tongue flicks) and b) if individuals exhibit preference

for related, compared to unrelated, individuals (measured via time spent in association). Several predictions are possible, based on this design. First, we predict that there will be differences in tongue flick rates directed towards related and unrelated individuals (measured in each arm of the maze). Second, if the pairing with related individuals observed in the wild is a result of active choice for more closely related individuals, we predict that individuals will spend more time associated with more related individuals of the opposite sex compared to less related individuals. Alternatively, if pairing with a related individual is the result of constraints, then we predict that individuals will spend less time associated with more related individuals of the opposite sex or will spend equal amounts of time with both potential mates. Finally, as trade-offs between the benefits and costs of inbreeding are suggested to differ between males and females due to unequal patterns of reproductive investment (Pizzari et al. 2004; Kokko and Ots 2006; Reid et al. 2015), we predict that the responses will be stronger in females compared to males.

Materials and Methods

Study Species

Liopholis whitii is a medium-sized (up to 100 mm snout-vent length (SVL)) viviparous lizard found throughout a broad altitudinal (0–1600 m) and ecological (coastal heaths, grasslands, and forests) range in south-eastern Australia. We used *L. whitii* from the east coast of Tasmania, Australia (42°57′S, 147°88′E). In this population, males and females are sexually monomorphic, become reproductively mature at approximately 3 years and display an overall lifespan of 9–10 years (GMW unpublished data). Reproduction occurs annually, with mating during the austral spring (September–October) and gestation spanning 3–4 months (While et al. 2009a). Recent work from natural populations of *L. whitii* showed that there is substantial variation in relatedness within populations, creating opportunities for both inbreeding preference and avoidance (Chapple and Keogh 2005; While et al. 2014).

Field methods

We captured 71 adult lizards (SVL ≥ 75 mm) at the beginning of the Austral autumn (March) in 2008. All individuals were captured from an area of approximately 500m x

500m, using 'mealworming' and noosing techniques. Once captured, individuals were measured for weight (± 0.1 mg) and length (SVL and total length; ± 0.5 mm), and sex was determined via eversion of the hemipenes. Each individual had a portion of its tail clipped and stored in 70% ethanol for later DNA extraction and genetic analysis (see below).

Lizards were returned to the terrestrial ecology facilities at the University of Tasmania, Australia, where they were housed in a temperature- and light-controlled room. The room was set to ambient day length of 12 hours (Hobart, Tasmania, Australia) and each lizard was provided with a basking lamp (set on a timer to come on 1 hour after room lights and turned off 1 hour before rooms lights went off). All lizards were individually housed in identical rectangular plastic terraria (30 x 60 x 40 cm) and provided reptile bedding, a basking rock and the basking lamp at the one end, and a shelter at the opposite end of the terraria. Lizards were provided food (*Tenebrio* larvae) and water *ad libitum*.

We caught lizards prior to hibernation. As the mating season begins soon after emergence from hibernation this ensured that no individuals were paired, or had a mate, prior to the mate discrimination trials. This required us to hibernate lizards for the duration of winter in the laboratory. Following a two week settlement period, we began shortening the ambient day length by one hour each week (30 minutes in the morning and 30 minutes in the afternoon), which roughly corresponds with natural conditions (Hobart, Tasmania, Australia). Basking lights were also reduced by the corresponding time. After 6 weeks (once day length had reached a length of 6 hours per week) we switched basking lights off and maintained lizards under ambient day light only for a week before switching all room lights off. Individuals were then kept under these conditions throughout the duration of winter (mid-May to late-July). During this time, the laboratory temperature was maintained at 5^{o}C (corresponding with average temperatures in winter in Tasmania). To maintain a low metabolic rate during hibernation lizards were only provided with water (no light source). In mid-July, overhead lights were switched back on for 6 hours a day. After a week, basking lights were also switched on to the corresponding day length and food was provided as above. Ambient and basking light day length was then increased by 2 hours per day until ambient day length reached 12 hours per day.

Experimental Design and Data collection

Experimental trials were carried out in late August, which corresponds to the beginning of the spring mating season for this species (GMW pers. obs.). Receptivity of lizards was confirmed by allowing a number of individuals (n=10) to mate following completion of the mate discrimination trials. In all cases mating behaviour, including tongue flicks, chasing and mounting, was observed.

To test discrimination between mates we used Y-mazes. These were constructed from opaque Polyvinyl chloride sheets and cut to 90 cm in length, with a main arm of 45 cm and two arms of 45 cm each and covered with fitted clear perspex lids. Both arms and the main chamber were 10 cm wide. A fan was attached at the end of the main chamber which drew air from the two arms, facilitating the distribution of scent, from each arm, throughout the maze. Partitions were placed at the end of each arm to prevent the focal lizard seeing and coming into physical contact with either potential mate. A 1 cm space underneath each partition allowed air flow from the potential mates to the focal animal, facilitating olfactory communication.

Prior to the experimental treatments, individuals and potential mates were kept in separate temporary plastic terraria. All experiments were run during the day in a temperature controlled room set at 18°C. Two basking lamps (40 W globes) were placed above either end of the Y-maze to provide heat to the subject lizards. The base of the maze was lined with reptile bedding and paper was attached at a height of 3 cm along the walls of the Y-maze to prevent individuals from responding to their reflection.

We ran 21 replicates for each sex separately (yielding 42 replicates in total). Each individual was presented with a choice of two potential mates, which were allocated to one of the two arms of the Y-maze, with one arm containing a closely related mate and the other a less related mate. The arm of the maze containing the related and unrelated potential mate was randomized for each test. Potential mates were assigned from a spectrum of pairwise relatedness values estimated for all individuals based on prior genetic analysis (see below). The average relatedness of individuals designated as closely-related was 0.16 ± 0.04 and 0.20 ± 0.02 for males and females respectively (all data reported as means ± 1SE), and for individuals designated as less-related was -0.18 ± 0.03 and -0.14 ± 0.02 for males and females respectively (see Table A6.1 for full range of relatedness values between treatments). This range of relatedness values closely corresponds to the range of mates available in the wild (-0.22 to 0.41 for social and

extra-pair mates; see While et al. 2014). Furthermore, the level of relatedness for the more closely-related individual (~0.20) is in accordance with much of the theory that suggests that optimal inbreeding is associated with slightly more distantly related kin as opposed to immediate relatives (Bateson 1982, 1983). The mean difference between related and unrelated potential mates was 0.31 ± 0.04 for males and 0.33 ± 0.03 for females. The closely related and less related potential mates were similar in body size (males; closely related: 79.32 ± 0.72 mm *vs.* less related: 80.32 ± 0.81 mm, females; closely related: 76.26 ± 1.06 mm *vs.* less related: 77.82 ± 1.18; overall effect of treatment across the sexes: $F_{1,87} = 1.79$, P = 0.18) and in their capture distance from the focal individuals (closely-related = 83.22 ± 6.95 m *vs.* less-related = 83.71 ± 6.41 m; $F_{1,87} = 0.27$, P = 0.96). The latter result allowed us to separate out mate choice based on relatedness *vs.* that based on familiarity.

Focal individuals were placed at the base of the main chamber of the Y-maze, behind a partition, and allowed to acclimatize to the maze for 5 minutes. The partition was then lifted, and the individual released into the Y-maze for 30 minutes. During each trial a number of behaviours were recorded by a single observer (GB). The experimenter was not blind to the experimental replicates, but as we had potential predictions for responses in both directions this should not have entered any bias into the results. First, we recorded the number of tongue flicks the focal individual performed in the main chamber and in each arm. Tongue flicks are a common means of determining the extent of discrimination and preference in lizard studies (e.g. Pernatta et al. 2009; Hews et al. 2011; Lopez and Martin 2012; Scott et al. 2015; Heathcote et al. 2016). However, as there are a number of problems associated with functionally interpreting differential tongue-flicking rates (see Heathcote et al. 2014), we use tongue flick rates here as evidence for discrimination rather than mate choice or preference. Second we recorded the latency it took the focal individual to enter each arm of the maze (in seconds). Finally, we recorded the total amount of time the focal individual spent associated with either stimulus individual in a given arm (in seconds). Social associations between males and females are commonly used as a means to study potential mating decisions in lizards (see Martín and López 2000; López et al. 2002; López and Martín 2005). The experimenter was not blind to the experimental replicates, but as we had predictions for responses in both directions (see introduction) this should have limited to potential to enter bias into the results.

At the completion of each treatment, both the focal individual and the potential mates were removed from the maze, the Y-maze was cleaned with 70% ethanol and dried. Unsuccessful (n = 6) trials occurred when the focal individual remained in the start area for 20 minutes; re-run replicates were conducted the following day with the same focal and subject individuals. At the completion of the experiment all lizards were returned to their site of capture.

Molecular and relatedness analysis

Microsatellite genotyping

DNA was extracted from tail tip samples using the hexadecyltrimethyl ammonium bromide (CTAB) protocol. All individuals used in this study were genotyped for four tetranucleotide microsatellite loci (EST1, EST2, EST4, EST12; Gardner et al. 1999) and two di-nucleotide microsatellite loci (Trl12, Trl 28; Gardner et al. 2008). These loci are highly variable and informative and conform to the expectations of Hardy–Weinberg equilibrium (this study; see also While et al. 2009a; Chapple and Keogh 2005). Results were analysed with Beckman Coulter CEQ8000 Genetic Analysis software.

Estimates of Genetic Relatedness

The program COANCESTRY (Wang 2011) was used to estimate pairwise relatedness between males and females. This program calculates the Queller and Goodnight (1989) index of relatedness (R). Standard errors of R estimates were obtained by jack-knifing over the six loci. A single relatedness analysis was performed on all individuals to calculate estimates of pairwise relatedness between all males and females. From this we assigned two potential mates for each individual, one closely related and one distantly related to the focal individual. Potential mates were assigned from a spectrum of pairwise relatedness values estimated for all individuals (Table A6.1).

Statistical Analysis

For count variables (number of tongue flicks) and binary responses (choice of treatment arm) we ran generalized linear models fit with Poisson and binomial error distributions in the 'lme4' package (Bates et al. 2015), respectively. For time spent in each treatment arm we ran a beta regression using the 'betareg' package (Grün et al. 2012), taking the proportion of total trial time spent in each arm as the response

variable. We began by including the main effects of sex and treatment and their pairwise interaction as predictor variables in all models. If interactions terms were non-significant ($P > 0.05$) we dropped them from the model and results from reduced models are reported. In total we ran five separate models with the response variables; (i) number of tongue flicks in the main chamber; (ii) number of tongue flicks in the treatment arms; (iii) latency to choose one of the treatment arms; (iv) choice of the first arm; and (v) time associated with the each potential mate in the treatment arms (see Table 1 for full model specification). For models in which multiple responses were included for the same focal individual (i.e. count of tongue flicks in each of the two treatment arms) we included a random effect of ID to account for repeated measures. For model (i) and (iii) we included the difference in relatedness between the two potential mates as a covariate in the models to control for variation in the degree of relatedness between potential mates. For our analysis of tongue flicks, we did not control for differences between individuals in the amount of time they spent in the main chamber or the arms because initial inspection of the data showed that there was no relationship between time spent and number of tongue flicks (main chamber: $F_{1,88} = 0.02$, $P = 0.88$; treatment arms: $F_{1,51} = 0.42$, $P = 0.52$). For the examination of the number of tongue flicks in the treatment arms we removed all responses in which subjects never entered the arm (i.e. in which time in the treatment arm = 0s). All statistical analyses were conducted using R version 3.3.0 (R Core Team 2013).

Table A6.1 Estimates of the mean of and range in relatedness between focal and the two subject (related and unrelated) individuals for both males and females

	Related			Unrelated		
	Mean ± SE	**Min**	**Max**	**Mean ± SE**	**Min**	**Max**
Male	0.16 ± 0.04	-0.11	0.68	-0.18 ± 0.03	-0.27	-0.02
Female	0.20 ± 0.02	-0.03	0.37	-0.14 ± 0.02	-0.25	0.06

Results

The difference in relatedness between the two stimulus animals did not significantly influence either the latency to enter an arm or the number of tongue flicks while in the main chamber for either sex (Table A6.2). Females performed more tongue flicks than males while in the main chamber before making a choice (Table A6.2; Fig. A6.1). Both sexes performed more tongue flicks in the treatment arm containing the less related potential mate (Table A6.2; Fig. A6.2).

Both sexes were more likely to choose the arm containing the more-closely related potential mate as their first choice (Table A6.2). A marginally non-significant treatment-by-sex interaction indicated a trend for males to choose the related arm first more often than females (p = 0.07; Table A6.2). The proportion of time spent in each treatment arm showed the same pattern (Table A6.2; Fig. A6.3); the model reported a main effect of treatment, indicating a greater proportion of time spent in the arm containing the more closely-related potential mate with a marginally non-significant treatment by sex interaction.

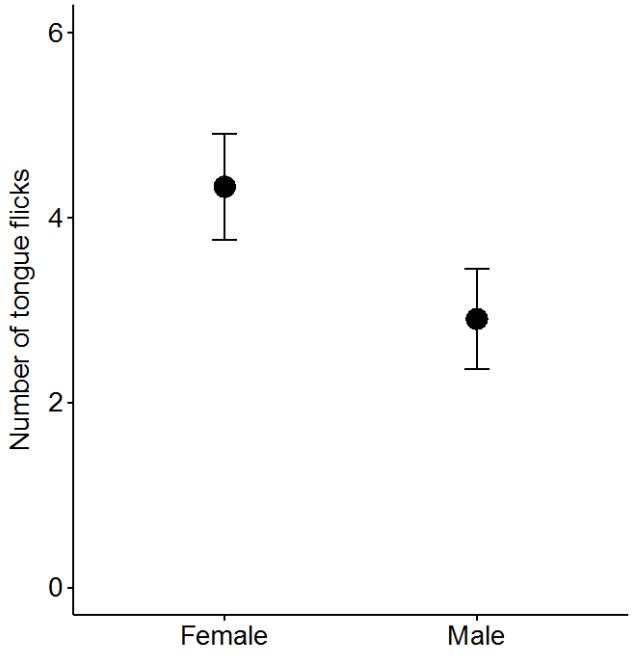

Figure A6.1. Mean (±SE) number of tongue flicks performed by males and females in the main chamber of the Y-maze.

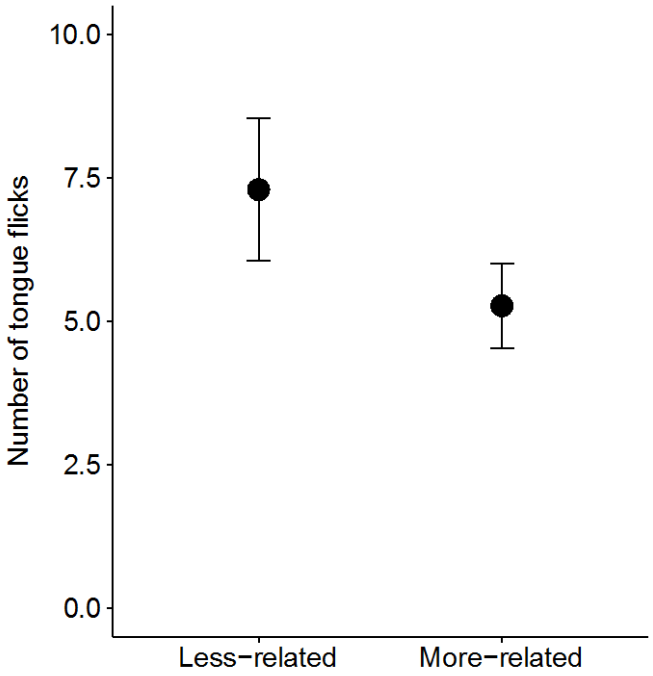

Figure A6.2. Mean (±SE) number of tongue flicks performed in the less related and more related treatment arm of the Y-maze for males and females combined.

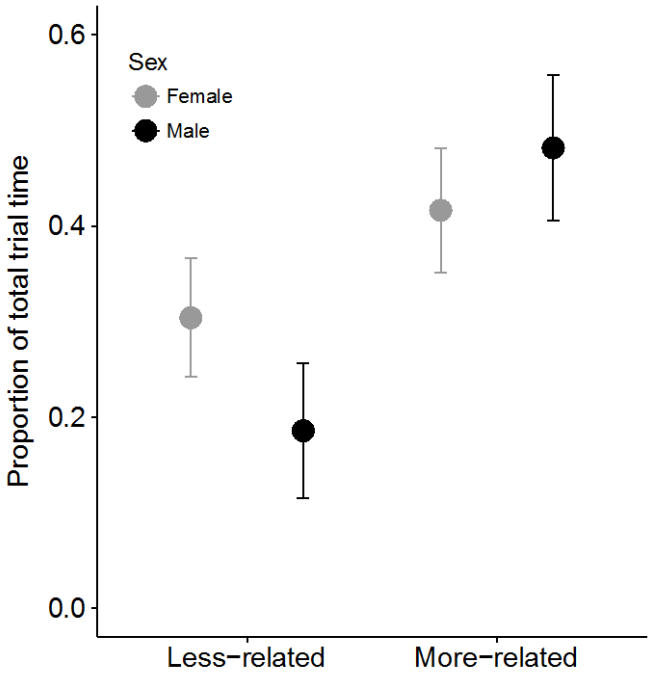

Figure A6.3. Proportion (±SE) of total trial time spent in the less related and more related treatment arm for males and females.

Table A6.2 The influence of sex, treatment and the difference in relatedness between potential stimulus choices on discrimination and time association behaviours. Results for main effects are reported from reduced models after non-significant interaction terms were dropped (significance of these terms shown). Dashes represent terms that were not included in the specified model. * indicates that sex was unable to be measured as a main effect in this model because every individual of both sexes made a first choice

Variable	Sex	Treatment	Difference in relatedness	Sex x Treatment
Tongue flicks (main chamber)	**$\chi^2 = 7.06, P < 0.01$**	-	$\chi^2 = 1.24, P = 0.27$	-
Latency	$\chi^2 = 0.02, P = 0.89$	-	$\chi^2 = 1.05, P = 0.31$	-
Tongue flicks (treatment arm)	$\chi^2 = 0.08, P = 0.77$	**$\chi^2 = 4.54, P = 0.03$**	-	$\chi^2 < 0.01, P = 0.96$
First choice	*	**$\chi^2 = 8.97, P < 0.01$**	**-**	$\chi^2 = 3.35, P = 0.07$
Time in arm	$\chi^2 = 0.147, P = 0.70$	**$\chi^2 = 5.91, P = 0.02$**	-	$\chi^2 = 3.27, P = 0.07$

Discussion

Previous research has identified non-random mating patterns of mate choice in this population of *L. whitii*. Males and females pair with closely related individuals to a greater extent than expected by chance (While et al. 2014). This has been suggested to be a consequence of limited natal dispersal resulting in substantial genetic structuring which, in combination with low breeder turn over, constrains social mate encounter rates and thus biases pairing towards relatives (While et al. 2014). However, recent theoretical, and growing empirical, research has cautioned against such interpretations and has, instead, suggested that such patterns may be the result of active preference for closely related individuals (Kokko and Ots 2006; Oh 2011). Here, we confirm that male and female *L. whitii* distinguish between individuals based on their relatedness and, furthermore, both sexes preferentially associate with more closely related members of the opposite sex.

Tongue flicks are commonly used to measure discrimination in squamate reptiles (Pernatta et al. 2009; Hews et al. 2011; Lopez and Martin 2012; Scott et al. 2015; Heathcote et al. 2016) as olfactory communication is the primary means of social communication in this taxon. Consistent with our prediction, both sexes showed a difference in the number of tongue flicks directed towards related *vs.* unrelated potential mates. Specifically, individuals tongue flicked more when in the arm with a less related potential mate compared to the arm with a more related potential mate. This result suggests an ability to discriminate between potential mates based on their genetic similarity, which is consistent with research on a number of other species in the *Egernia* group (e.g. Main and Bull 1996; Bull et al. 2001; O'Connor and Shine 2006). In further support of this, we also found that individuals biased their choice of arms, both in terms of their initial preference and the amount of time spent in the arms. Given that we restricted all other modes of discrimination, these results strongly suggest an ability to discriminate between related and unrelated individuals based on scent in *L. whitii* (see also While et al. 2009b, 2014).

The strong difference in the amount of time spent associated with related *vs.* unrelated potential mate, when combined with patterns of mate choice from the natural population (While et al. 2014), suggests that mate choice with respect to relatedness may be an active rather than passive process in this population. This interpretation is

consistent with a growing body of literature demonstrating a positive preference for pairing with kin across a range of taxa (e.g. Kleven et al. 2005; Thünken et al. 2007; Langen et al. 2011; Wang and Lu 2011; Robinson et al. 2012). Inbreeding preference is predicted to emerge whenever the inclusive fitness benefits outweigh the costs of reduced offspring quality as a result of inbreeding depression (Waser et al. 1998; Parker 2006; Szulkin et al. 2013). Research from wild populations of birds have begun to show that individuals can gain substantial inclusive fitness benefits from inbreeding, despite reduced offspring quality (Reid et al. 2015). To fully understand the extent to which mate choice in *L. whitii* is a result of passive constraints associated with population viscosity or an active preference for mating with related individuals, similar long-term data on the consequences of inbreeding and outbreeding for both males and females are required. Such an approach also has the potential to shed light on the heritability of inbreeding preference, and thus the degree to which inbreeding systems might evolve in response to selection (see Wolak and Reid 2016). It may also allow us to examine the extent to which the benefits of inbreeding differ between within-pair and extra-pair copulations. Theoretical models have suggested that optimal inbreeding strategies can differ substantially between mating systems, both within and among populations (Lehtonen and Kokko 2015). In our natural population, individuals pair with closely related individuals but pursue/accept extra-pair copulations from less related individuals (While et al. 2014). If the patterns of social mate choice observed in our natural population are the result of a preference for closely related individuals, we require an explanation for why individuals choose less related individuals as extra-pair partners. Speculation on this topic is outside the scope of this paper but provides an exciting potential direction for future research.

We also found that the extent of preference for related individuals differed, to some extent, between the sexes. Specifically, males tended to exhibit a stronger preference for more closely related partners than did females both in terms of initial choice and time spent in association. While these differences did not reach statistical significance, potentially due to limited sample size, they are suggestive of potential sexual conflict over inbreeding. Producing inbred offspring is expected to have increased fitness consequences for females compared to males due to unequal patterns of reproductive investment (Pizzari et al. 2004; Parker 2006; Duthie and Reid 2015). Therefore, females should exhibit a greater plasticity in their mate preferences in

response to social context compared to males, potentially resulting in weaker overall effect sizes for inbreeding preference.

One caveat to our study is that our experimental design relies on the assumption that our association data represent a mating preference (see methods for studies that have used this approach in the past). An alternative interpretation is that these results represent a more general preference for more closely related individuals. This could be to avoid aggressive behaviour as relatedness can moderate aggression between individuals (Carazo et al. 2014; Griesser et al. 2015) and previous research on mate choice in lizards has shown a preference for less threatening males in the laboratory (opposite to the patterns observed in the wild; Carazo et al. 2011) or because clusters of closely related individuals represent a cue to that individual's home range. While these possibilities cannot be ruled out without additional experimental trials (e.g. examining the extent to which aggression between individuals co-varies with relatedness, intra *vs.* inter sexual preference trials, staged mating trials), low levels of inter-sexual aggression compared to intra-sexual aggression observed in both natural and experimental conditions (While et al. 2009a; Halliwell et al. 2016) combined with the consistency of preference observed in the wild and the laboratory suggest these interpretations are unlikely. Alternatively, a preference for kin may be explained by a broader function for kin recognition as a result of parental care/tolerance which is important in this species (e.g. While et al. 2009b). Nonetheless, regardless of the evolutionary origin of a mechanism for kin recognition in this system, this could be co-opted to serve to enable distinguish between related and unrelated individuals in a mating context, as suggested by our previous work (While et al. 2014; this study).

In summary, our results suggest that mate choice for related individuals within our system is the result of an active preference for related individuals, rather than a constraint on optimal outbreeding as a result of population viscosity. However, in order to understand the extent to which such preferences for inbreeding are adaptive, we must integrate these findings with the long-term fitness costs and benefits of inbreeding/outbreeding (see Reid et al. 2015). At a taxonomic scale, these data can be combined with patterns of mate choice with respect to inbreeding observed across the broader *Egernia* group (within which *L. whitii* resides). The *Egernia* group consists of species which vary in key ecological, social (varying from solitary to large communal family groups) and life-history characteristics (see Chapple 2003; While et al. 2015)

that have the potential to influence the trade-off between the inclusive fitness benefits and the costs of inbreeding. These species also exhibit different inbreeding strategies at both the social and extra-pair level (with inbreeding avoidance, tolerance and preference observed; Bull and Cooper 1999; Gardner et al. 2001; Stow and Sunnocks 2004; Chapple and Keogh 2005). This has the potential to provide an outstanding comparative framework in which we can explore the ecological and life history conditions under which animals make mating decisions related to inbreeding. Such an approach will allow us to move away from asking whether a population exhibits inbreeding avoidance or tolerance and towards a more holistic approach which focuses on the context dependent nature of the costs and benefits of inbreeding. Ultimately this will enable us to identifying the ecological, social and life-history traits which are likely to predict when individuals should avoid, tolerate or prefer relatives as mates (Szulkin et al. 2013).

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable institutional and/or national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the University of Tasmania, Australia.

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